

Influence of sodium chloride and its combination with Fe (II) or Cu (II) on the oxidative alteration of butter model systems

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RESUMEN

Influencia del cloruro sódico y sus combinaciones con Fe(II) o Cu(II) sobre la alteración oxidativa de sistemas modelos de grasa de leche.

Se estudia el efecto de la concentración de NaCl (10.7, 21.4, 46 y 66 g kg⁻¹) y combinaciones de NaCl (10 ó 20 g kg⁻¹) y Fe(II) o Cu(II) (25 or 50 µgg⁻¹) en sistemas modelo de dispersiones de agua en grasa de leche (WDIBFS) durante el almacenamiento. La evaluación de la oxidación se realiza por medio de los índices de peróxidos, p-anisidina y TOTOX. Los resultados indicaron que los niveles de NaCl de 10.7, 21.4 y 46 g kg⁻¹ retardaron significativamente la oxidación durante los 6 meses de almacenamiento, mientras que la concentración más elevada de 66 g kg⁻¹ mostró un efecto prooxidante. La sal aumentó significativamente ($p \leq 0.05$) el efecto prooxidante del Cu(II) en todas las combinaciones ensayadas, siendo el efecto directamente proporcional a la concentración de sal. Por otra parte, la adición de NaCl aumentó significativamente el efecto prooxidante del Fe(II) aunque no existieron diferencias significativas entre los dos niveles de sal (10 y 20 gkg⁻¹). Los iones Fe(II) fueron más efectivos como prooxidantes que los iones Cu(II) en los sistemas modelo libres de sal. Sin embargo, la adición de sal eliminó las diferencias observadas entre ambos iones.

PALABRAS-CLAVE: Autoxidación - Cu(II) - Fe(II) - Grasa de leche - Índice de peróxidos - Índice de p-anisidina - NaCl.

SUMMARY

Influence of sodium chloride and its combination with Fe (II) or Cu (II) on the oxidative alteration of butter model systems.

The effect of the incorporation of different levels of NaCl at 10.7, 21.4, 46 and 66 g kg⁻¹, and the combinations of NaCl (10 or 20 g kg⁻¹) and Fe(II) or Cu(II) (25 or 50 µgg⁻¹) in model systems of water dispersion in butter fat (WDIBFS) during storage was investigated by measuring peroxide value, p-anisidine index and TOTOX. The results indicated that NaCl at 10.7, 21.4 and 46 g kg⁻¹ levels had a significant retarding effect against fat oxidation during 6 months of storage, while the higher concentration of 66 g kg⁻¹ salt showed a significant prooxidant effect. NaCl significantly ($p \leq 0.05$) increased the prooxidant effect of Cu(II) in all combinations, whereby the increase was directly proportional to the salt concentration. On the other hand, the addition of NaCl significantly increased the prooxidant effect of Fe(II) ions with no significant differences between the two salt levels (10 and 20 gkg⁻¹). Ferrous ions were more effective as prooxidant than Cu(II) ions in the salt free WDIBFS. However, the addition of salt almost canceled the observed differences of the prooxidant influence of Fe(II) and Cu(II) ions.

KEY-WORDS: Autoxidation - Butter fat - Cu(II) - Fe(II) - NaCl - Peroxide value - p-anisidine index.

1. INTRODUCTION

The oxidative alteration of fats and oils and fatty foods is of great importance since it affects the quality of foods due to the development of rancidity (Frankel, 1985; Grosch, 1987), loss of nutritional value and formation of toxic compounds (Eriksson, 1987; Kubow, 1992; Esterbauer, 1993).

Lipid oxidation is affected by many factors including elements such as iron and copper and NaCl. Ellis *et al.* (1970) demonstrated that the addition of salt at different levels produced a protective effect against the oxidation of fat in a hydrated gel and a prooxidant effect in the freeze-dried gel. Mabrouk and Dungan (1960) studied the influence of oxygen absorption of NaCl in emulsions of methyl linoleate in water, and they observed progressive inhibition related to salt concentration. Tichivangana and Morrissey (1985) reported that ferrous ions induced a higher level of lipid oxidation than cupric ions in cooked meat. Salih *et al.* (1989) found that (salt, Fe(II)) and (salt, Cu(II)) combinations showed higher levels of lipid oxidation in turkey meat than Fe(II) or Cu(II) alone. Farouk *et al.* (1991) reported that the addition of ferrous ions and /or salt resulted in a marked increase in lipid oxidation in both uncooked and cooked ground beef during storage at 8-9°C. It has been reported that the addition of NaCl showed an increase in lipid oxidation in poultry meat (King and Earl, 1988). Apgar and Hultin (1982) found that the addition of salt increased the enzymatic oxidation in fish muscle stored at -12°C. On the other hand, Salih (1986) reported that metal impurities, and not the salt, increased lipid oxidation.

Most of the works that investigated the effect of NaCl or table salt and their combinations with metal ions on lipid oxidation were carried out on oil / water emulsions or on meat. However, no published works have been found that elucidate the effect of NaCl and its combinations with mineral ions on the fat oxidation in pure water/oil emulsions. Therefore, this study aimed to investigate the effect of NaCl and its combinations with ferrous or cupric ions on fat oxidation using fat/water model systems.

2. MATERIAL AND METHODS

2.1. Chemical reagents

Sodium chloride (BDH, UK), ferrous chloride (Fisher, New Jersey, USA), copper sulfate (Merck, Germany), and all other chemicals or solvents used in the analysis were of analytical grades.

2.2. Anhydrous butter fat (ABF)

ABF was prepared in the laboratory following a traditional method (Amr, 1991): Durum wheat bulgur grits (75 g kg^{-1} butter) were added to t unsalted cow's butter, prepared from soured milk and obtained from a local producer. The mixture was then heated to a temperature of 117°C , which was found to be the temperature at which the bulgur starts to gather and precipitate at the bottom of the container. Heating was continued until a clear butter oil was obtained (about 15 min). The hot liquid of ABF was decanted and filtered through a piece of cheesecloth, poured into glass jars and left to solidify.

2.3. Butter fat model systems containing sodium chloride

Five portions of ABF (80g each) were warmed to a temperature slightly below its melting point ($33\text{-}35^\circ\text{C}$). 20 ml of sterilized NaCl solutions of 50, 100, 200 g kg^{-1} and a saturated one were added separately to 4 of the ABF portions, while 20 ml of sterilized distilled water was added to the fifth portion (control). The mixtures were then stirred thoroughly using a Hamilton homogenizer (Scovil, USA) for 3 min. NaCl concentrations based on the total weight of these mixtures were 0, 10.7, 21.4 and 66 g kg^{-1} (Table I). Each mixture was divided into 5 portions (18-20 g), which were then poured into sterilized light protected polystyrene cups (200 g capacity), heat sealed using aliminum laminate lids and stored at room temperature. Samples were analyzed after 0, 1, 3, 4 and 6 months of storage.

2.4. Butter model system containing combinations of NaCl and Fe(II) or Cu(II)

Sterilized aqueous solutions of NaCl at two concentrations (50 g kg^{-1} and 100 g kg^{-1}) or distilled water containing ferrous ions (FeCl_2) or cupric ions (CuSO_4) were added to ABF samples heated to temperatures slightly below their melting points ($32\text{-}35^\circ\text{C}$) to prepare butter fat model systems which were composed of 80 g of ABF and 20 g of the aqueous phase. The concentrations of NaCl based on the total weight of these model systems were 0, 10 or 20 g kg^{-1} , while the concetrations of Fe (II) and

Cu (II) ions were 0 or 25 or $50 \mu\text{g g}^{-1}$ (Table I). The mixtures were then mixed thoroughly using a Hamilton Beach (Scovil, USA) homogenizer for 4 minutes. Six samples (18-20 g each) from each model system were filled in light protected polystyrene cups (200-g capacity) and then heat sealed using aluminum laminate lids. Samples were taken for analysis after 0, 4, 8, 12, 16, 20 days of storage at room temperature.

2.5. Microscopy of water in butter fat dispersion

Small portions of the model systems were mixed with a water-soluble dye and minute amounts of these systems were smeared on glass slides, covered with glass covers and examined under a microscope (Olympus Opticals, Japan) using 400x and 1000x magnification.

2.6. Chemical analysis

Lipid oxidation was evaluated for peroxide value, p-anisidine index and TOTOX. Peroxide value (PV) was carried out following the AOAC (1980) iodometric method (number 28:002) and expressed as $\text{mEq O}_2 \text{ Kg}^{-1}$ total model system. p-Anisidine index (p-ANIS) was carried out following the NGD C35 spectrophotometric method (1979). TOTOX is a value that tends to indicate the total oxidation of a sample, which is equivalent to $2 \text{ PV} + \text{p-ANIS}$ (Rossell, 1987).

2.7. Statistical analysis

The analysis of the variance (ANOVA) was performed using the Statistical Analysis System (1985) program. Significant differences between means were determined by Duncan's multiple-range tests.

3. RESULTS AND DISCUSSION

3.1. Description of the system and prevailing conditions for oxidation

The microscopy of the systems revealed that all preparations were water in fat dispersion, since the colored aqueous phase appeared as colored droplets uniformly distributed in the fat mass. In these systems, all ions are supposed to be in the aqueous phase and their influence would be in the interface of the two phases. It is clear that the fat surface area surrounding the aqueous droplets is equal to that of the aqueous phase and accordingly the fat surface is not a limiting factor for the studied effect.

Table I
The (NaCl, Fe(II)) and (NaCl, Cu(II)) combinations that were prepared to evaluate the effect of NaCl on fat oxidation and the prooxidant activity of ferrous and copper ions

Sample	NaCl (g kg ⁻¹)	Fe(II) (µg g ⁻¹)	Cu(II) (µg g ⁻¹)
1	-	-	-
2	10.7	-	-
3	21.4	-	-
4	46	-	-
5	66	-	-
6	-	25	-
7	-	50	-
8	-	-	25
9	-	-	50
10	10	25	-
11	10	50	-
12	20	25	-
13	20	50	-
14	10	-	25
15	10	-	50
16	20	-	25
17	20	-	50

On the other hand, the samples, which weighed about 18 g, kept cups having diameters of 7 cm, capacities of about 200 g, and sealed, indicate that oxygen enclosed inside the cups was abundant and not a limiting factor for fat oxidation. Furthermore, since the surface area of the sample relative to its volume was high (the diameter of the cup was 7 cm and the thickness of the fat dispersion was about 2 mm), it is assumed that the diffusion rate in this layer was not a limiting factor either for the oxidation process. Thus, it can be concluded that the observed differences in fat oxidation are due to the effect of the added ions and their concentrations.

3.2. Effect of different levels of NaCl on fat oxidation in butter fat/water model system during long-term storage

The results of the effect of different levels of NaCl on fat oxidation in water / butter fat model systems (WDIBFS) during storage as evaluated by peroxide value (PV) are reported in Table II. From the data it is quite clear that the addition of NaCl at concentrations of 10.7, 21.4 and 46 g kg⁻¹ seemed to retard fat oxidation as measured by peroxide value. The first two NaCl concentrations showed similar effects and they were more effective than the third one (46 g kg⁻¹)

Table II
Peroxide value (mEq O₂ kg⁻¹ total model system) of water dispersion in butterfat model systems treated with different levels of NaCl during storage at room temperature (1,2)

Storage time (months)	Level of the added NaCl g kg ⁻¹				
	0	10.7	21.4	46	66
0	ND	ND	ND	ND	ND
1	1.3 ^a	1.3 ^a	1.1 ^a	1.3 ^a	1.5 ^a
3	2.5 ^b	2.4 ^b	2.3 ^b	2.4 ^b	15.7 ^a
4	41.4 ^b	14.2 ^d	16.4 ^{cd}	18.5 ^c	76.7 ^a
6	92.1 ^b	31.1 ^d	32.4 ^d	44.7 ^c	161.1 ^a

¹Mean values of three replicates.

²Means with different superscript within the same row are significantly different.
ND: not detected.

Table III
Effect of NaCl / Cu²⁺ combinations on peroxide value (PV), p-Anisidine index (p-ANIS) and TOTOX in butter fat model systems

Treatment	PV ^a										p-ANIS ^a										TOTOX ^a									
	Time of storage (days)					Time of storage (days)					Time of storage (days)					Time of storage (days)					Time of storage (days)									
	0	4	8	12	16	20	0	4	8	12	16	20	0	4	8	12	16	20	0	4	8	12	16	20						
Cu ²⁺ (25 µgg ⁻¹)	ND ^{c,b}	0.8 ^c	1.4 ^c	2.5 ^c	3.1 ^c	5.7 ^c	0.3 ^c	0.4 ^c	0.4 ^c	1.0 ^c	2.5 ^c	3.8 ^c	0.3 ^c	0.3 ^c	2.0 ^c	3.2 ^c	6.0 ^c	8.7 ^c	0.3 ^c	2.7 ^d	6.4 ^d	17.1 ^d	48.9 ^d	15.2 ^c						
Cu ²⁺ (25 µgg ⁻¹), NaCl (10 gkg ⁻¹)	ND ^c	1.1 ^{cd}	3.0 ^d	6.5 ^d	15.6 ^d	33.9 ^d	0.3 ^c	0.5 ^d	0.4 ^d	4.1 ^d	8.7 ^d	17.0 ^d	0.3 ^d	0.3 ^d	2.7 ^d	6.4 ^d	17.1 ^d	48.9 ^d	0.3 ^d	2.7 ^d	6.4 ^d	17.1 ^d	48.9 ^d	60.8 ^d						
Cu ²⁺ (25 µgg ⁻¹), NaCl (20 gkg ⁻¹)	ND ^c	1.8 ^d	5.7 ^e	13.6 ^e	28.9 ^e	56.4 ^e	0.3 ^c	1.3 ^e	1.8 ^e	7.3 ^e	16.4 ^e	30.7 ^e	0.3 ^e	0.3 ^e	4.9 ^e	13.2 ^e	34.5 ^e	74.2 ^e	0.3 ^e	4.9 ^e	13.2 ^e	34.5 ^e	74.2 ^e	143.5 ^e						
Cu ²⁺ (50 µgg ⁻¹)	ND ^{c,f}	4.4 ^c	12.5 ^c	-	-	-	0.3 ^c	2.5 ^c	7.5 ^c	-	-	-	0.3 ^c	0.3 ^c	11.3 ^c	32.5 ^c	-	-	0.3 ^c	11.3 ^c	32.5 ^c	-	-	-						
Cu ²⁺ (50 µgg ⁻¹), NaCl (10 gkg ⁻¹)	ND ^c	18.7 ^d	70.5 ^d	-	-	-	0.3 ^c	18.3 ^d	57.7 ^d	-	-	-	0.3 ^c	0.3 ^c	55.7 ^d	197.7 ^d	-	-	0.3 ^c	55.7 ^d	197.7 ^d	-	-	-						
Cu ²⁺ (50 µgg ⁻¹), NaCl (20 gkg ⁻¹)	ND ^c	30.5 ^e	88.9 ^e	-	-	-	0.3 ^c	43.2 ^e	85.9 ^e	-	-	-	0.3 ^c	0.3 ^c	104.2 ^e	267.7 ^e	-	-	0.3 ^c	104.2 ^e	267.7 ^e	-	-	-						

^aMean of three replicates

^bMeans within the same column with different letters are significantly different (p ≤ 0.05).

Table IV
Effect of NaCl /Fe²⁺ combinations on peroxide value (PV), p-Anisidine index (p-ANIS) and TOTOX in water dispersion in butter fat model systems.

Treatment	PV ^a						p-ANIS ^a						TOTOX ^a					
	0	4	8	12	16	20	0	4	8	12	16	20	0	4	8	12	16	20
Fe ²⁺ (25 µg g ⁻¹)																		
	ND ^{c,b}	1.2 ^c	2.2 ^c	4.1 ^c	7.5 ^c	15.3 ^c	0.3 ^c	0.4 ^c	0.7 ^c	1.9 ^c	5.0 ^c	8.2 ^c	0.3 ^c	2.8 ^c	5.1 ^c	10.1 ^c	20.0 ^c	3
Fe ²⁺ (25µg g ⁻¹), NaCl (10 gkg ⁻¹)	ND ^c	1.0 ^c	3.1 ^d	6.9 ^d	17.8 ^d	48.3 ^d	0.3 ^c	0.4 ^c	1.9 ^d	4.2 ^d	9.5 ^d	23.5 ^d	0.3 ^c	2.4 ^c	8.1 ^d	18.6 ^d	45.1 ^d	120.1 ^d
Fe ²⁺ (25µg g ⁻¹), NaCl (20 gkg ⁻¹)	ND ^c	ND ^c	1.6 ^d	3.5 ^d	7.6 ^d	19.6 ^d	50.4 ^d	0.3 ^c	0.5 ^c	2.2 ^d	4.5 ^d	9.5 ^d	25.3 ^d	0.3 ^d	3.7 ^d	9.2 ^d	19.7 ^d	48.7 ^d
Fe ²⁺ (50µgg ⁻¹)																		125.7 ^d
	ND ^{c,f}	8.3 ^c	27.5 ^c	-	-	-	0.3 ^c	3.7 ^c	18.5 ^c	-	-	-	0.3 ^c	20.3 ^c	73.5 ^c	-	-	-
Fe ²⁺ (50µgg ⁻¹), NaCl (10 gkg ⁻¹)	ND ^c	15.2 ^d	82.5 ^d	-	-	-	0.3 ^c	16.9 ^d	33.4 ^d	-	-	-	0.3 ^c	47.3 ^d	198.4 ^d	-	-	-
Fe ²⁺ (50µgg ⁻¹), NaCl (20 gkg ⁻¹)	ND ^c	16.6 ^d	83.8 ^d	-	-	-	0.3 ^c	17.9 ^d	34.4 ^d	-	-	-	0.3 ^c	51.1 ^d	202.0 ^d	-	-	-

^a Mean of three replicates

^b Means within the same column with different letters are significantly different (p ≤ 0.05).

in depressing the formation of peroxides. The peroxide values of the systems treated with these three NaCl concentrations after 6 months of storage were 33, 33 and 51 %, respectively of that of the control. In contrast, the addition of 66 g kg^{-1} NaCl accelerated fat oxidation. The PV of this sample after 6 months of storage was 180% of that of the control.

Ellis et al. (1970), reported that the incorporation of 23, 45 and 108 g kg^{-1} NaCl in a hydrated gel consisting of sodium carbomethoxy cellulose gum, lard and water (1:2:40) retarded lard autoxidation. However, in the freeze-dried gel, these concentrations accelerated lard oxidation. Chang and Watts (1950) investigated the influence of dry NaCl and its solutions at different concentrations on lard autoxidation. They concluded that NaCl at concentrations below 50 g kg^{-1} retarded, while dry NaCl and its solutions at concentrations of 100, 150 and 200 g kg^{-1} accelerated lard oxidation. Mabrouk and Dugan (1960) studied the level of methyl linoleate and linoleic acid oxidation in aqueous buffered solutions in the presence of different NaCl concentrations (0.54, 1.08, 2.6, 10.8 and $13.4 \text{ mole NaCl mole}^{-1}$ linoleic acid or methyl linoleate). They found that the degree of oxidation of methyl linoleate or linoleic acid was decreased with increasing concentration of NaCl. They attributed this result to the decrease of oxygen solubility in the buffered solutions with increasing NaCl concentration.

Based on the above findings, we assume that the prooxidant effect of NaCl at the highest concentration (66 g kg^{-1}) might be due to the low solubility of oxygen in the aqueous phase that should force the diffusing oxygen to accumulate in water/fat interface or increase the oxygen concentration in the oil phase enhancing fat oxidation. Another possible explanation is that the electrical conductivity increases proportionally with increasing ionized NaCl concentrations; i.e. the electrons' mobility would be enhanced, facilitating the oxidation process, which is defined as electron donation. However, the above interpretations cannot explain why the low levels of NaCl concentration do retard the autoxidation more than pure water.

3.3. Effect of NaCl on the prooxidant activity of Fe (II) and Cu (II) in water/ butter fat model systems (WBFMS)

The addition of Fe(II) or Cu(II) at 25 or $50 \text{ } \mu\text{g g}^{-1}$ fat to the water/fat systems resulted in the acceleration of autoxidation of fat at room temperature, which was significantly increased by doubling the amount of the added metal ions (Tables III & IV). These results are expected because it is well established that Fe (II) and Cu (II) ions do induce oxidation in butter at levels of 2 and $0.2 \text{ } \mu\text{g g}^{-1}$,

respectively (Belitz and Grosch, 1999). The data in Tables III and IV also show the effect of the interaction between cupric or ferrous ions and NaCl on fat oxidation. It is obvious that NaCl addition substantially increased the prooxidant action of the heavy metal ions, and in the case of cupric ions, the increase was significantly higher when NaCl concentration was doubled, whereas no such an effect was observed in the case of ferrous ions.

In the NaCl free model systems, ferrous ions were significantly ($P= 0.05$) more effective in the acceleration of fat oxidation than cupric ions. This might be attributed to the fact that Fe(II) ions decompose hydroperoxides at a greater rate generating higher amounts of free radicals than do Cu(II) ions (Belitz and Grosch, 1999). However, this behavior changed in the presence of NaCl, since the rates of fat oxidation in all NaCl, Cu(II) model systems were comparable to the corresponding NaCl, Fe(II) systems (Tables 3 & 4).

Harel (1994) suggested that NaCl increases the reaction rate of Fe(II) with hydroperoxides to produce free radicals. This suggestion may explain the observed effect of NaCl on the prooxidant activity of ferrous and cupric ions in the model systems used in the present study, and this effect was more pronounced in the case of cupric ions.

4. CONCLUSION

Sodium chloride at 10.7, 21.4 and 46 g kg^{-1} produced a protective effect on fat oxidation, while 6.6 g kg^{-1} showed prooxidant effect during long-term storage. Fe(II) or Cu(II) significantly increased the rate of fat oxidation in WDIBFS during storage. NaCl significantly increased the prooxidant effect of Cu(II) and Fe(II).

The results of this study along with those from previous studies demonstrate the complexity of the fat oxidation process even in model systems; i.e. it is inaccurate to make generalizations about the results obtained from one system to another. Differences such as fat type, colloidal status, presence of different solutes may be decisive in determining the rate of oxidation. Furthermore, the results obtained from different studies must be carefully interpreted and cannot usually be compared because they greatly depend on the analytical procedure to evaluate the extent and the end point of fat oxidation. Thus, it is recommended to investigate the effects of variable added common ingredients on fat stability of the products to optimize the formula of processed and fabricated foods in an attempt to avoid or reduce the use of chemical preservatives.

ACKNOWLEDGMENT

The authors thank Miss Nadia Al-dabbas for her careful technical assistance.

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Recibido: Mayo 2003
Aceptado: Abril 2004