POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

www.pan.olsztyn.pl/journal/
e-mail: joan@pan.olsztyn.pl

Pol. J. Food Nutr. Sci.
2008, Vol. 58, No. 1, pp. 91-94

INFLUENCE OF L-ASCORBIC ACID ADDITION ON THE CONTENT OF FATTY ACIDS IN BUTTER DURING STORAGE

Regina Borek-Wojciechowska

Chair of Quality Science,Technical University in Radom

Key words: fatty acids, L-ascorbic acid, storage

Butter continues to be readily purchased by consumers. The wealth of ingredients makes it a fat of exceptional nutritional value. Storage changes, particularly oxidation changes of butter fatty acids, are a major problem. Therefore, attempts are being made to slow these changes. This research presents an attempt at determining the effect of addition of L-ascorbic acid on fatty acid stability in butter during storage at 4°C. This study has shown that L-ascorbic acid affected contents of mono- and polyunsaturated acids in a statistically relevant way, but only after three and four weeks of storage.

INTRODUCTION

Milk lipid is an important fat in human nutrition. Owing to the unique composition of fatty acids, it is among the best absorbed edible lipids.

Milk lipid is an animal fat, though its significant portion is produced by the microflora in cow rumen, which makes its composition particularly complex [Przybojewska & Rafalski, 2003].

Adverse changes in butter are an important issue. Milk lipid alterations occur at every stage of butter production [Staniewski, 2000]. Oxidative changes pose a significant storage problem as they reduce nutritional value of the lipids. Therefore, antioxidants are added to lipids in order to slow oxidative changes. Antioxidants are specific chemical compounds which, when added in low quantities to food products, effectively counteract auto-oxidation processes in lipids and other readily oxidizing substances.

More generally, antioxidants are divided into: (1) antioxidants proper, reacting with radicals forming in auto-oxidation processes; and (2) synergents, mainly inactivating metallic catalysts or regenerating antioxidants properties [Pijanowski, 1974].

L-ascorbic acid is a well-known antioxidant with strong reducing and antioxidative properties, expressed in its reactivity to O_2, H_2O_2, $^{\bullet}OH$, peroxide radicals and singlet oxygen [Wefers & Sies, 1988].

In recent years, attempts have been made to introduce anti-oxidative substances of natural origin into butter [Ozcan & Ayar, 2003; Hadolin *et al*., 2005; Ayar *et al*., 2001; Żegarska *et al*., 1998].

Fatty acids are butter ingredients which are particularly sensitive to oxidation. According to Jensen *et al*. [1991], over 400 fatty acids were identified in milk lipid. Fatty acids play an important role in human body. Recently, attention has been drawn to their positive contribution. Butter is an excellent source of short-chain fatty acids. These acids are rapidly metabolised in the liver and do not contribute to fat tissue deposition, being also necessary for normal structure and functioning of large intestine epithelium. They can have a therapeutic effect on certain pathologies of the large intestine, *e.g.* a variety of inflammations or ulcerative inflammation of the large intestine. Germicidal, antiatherosclerotic and anticarcinogenic actions have also been attributed to these acids [Przybojewska & Rafalski, 2003]. In recent years, emphasis has been placed on food microelements, such as Conjugated Linolic Acid (CLA), that may contribute to improved health condition and counteract such civilisation diseases as tumors, diabetes and atherosclerosis. A great number of publications about CLA: 62 in 1998, 98 in 1999, and 126 in 2000, indicates a high interest in this acid. The authors attribute a range of beneficial properties to the Conjugated Linolic Acid: it counteracts arteriosclerosis, has bacteriostatic, antioxidative, anticarcinogenic properties (even in low quantities), and a positive influence on the immune system.

The aim of this study was to analyse the effect of L-ascorbic acid additives on changes in the total content of monounsaturated, polyunsaturated and saturated fatty acids of butter during storage.

MATERIAL AND METHODS

Commercial butter was used in the research. The butter was collected directly after manufacture. Half the butter was enriched with L-ascorbic acid: 15 mg acid/100 g butter – this was the maximum amount that did not produce ad-

Author's address for correspondence: Regina Borek-Wojciechowska, Chair of Quality Science, Technical University, ul. Chrobrego 31, 26-600 Radom, Poland; tel.: (48 36) 361 74 05; fax: (48 36) 361 74 51; e-mail: renia.woj@op.pl

verse organoleptic alterations. A water solution of the acid was introduced into the butter through homogenisation. The resultant samples were placed in commercial packaging and stored. Butter samples not fortified with L-ascorbic acid were also used in storage testing. All the samples were stored at a temperature of 4°C.

The butter samples, enriched and not enriched with L-ascorbic acid, were analysed chromatographically. Fat from the butter samples was extracted by melting, decantation and filtration through anhydrous sodium sulfate. Fatty acid methyl esters were prepared by fat transesterification with a KOH methanol solution according to IDF method [IDF Standard 184:1999]. The composition of fatty acids was determined by gas chromatography by means of HP 6890 N (Hewlett Packard, Palo Alto, CA) gas chromatograph including a flame-ionisation detector (FID) and a capillary column SUPELCOWAX 10 (30 m, ID 0.32 mm, film thickness 0.25 μm). The separation was carried out in a cycle of programmed temperatures: 65°C (1 min) to 180°C with an 8°C increase per minute. The temperatures of the injector and the detector were 230°C and 250°C, respectively. The flow rate of the carrier gas, helium, was 0.8 mL/ min, injector split (50:1).

Acids were identified by comparing retention times of the resulting peaks with peaks of reference methyl esters (Sigma Chemical Co., St.Louis, MO).

The percentage content of fatty acids was calculated in reference to the total fatty acid composition (weight percentage).

The content of acids was determined at 7-day intervals: the first determination was carried out after one day of storage. The results presented are an average of three repetitions. A statistical analysis of the results involved determination of functional models illustrating the course of changes, and verification of the hypothesis on the insignificance of a difference between two corresponding arithmetic mean values by means of a Student's t-test (at a significance level of α=0.05).

RESULTS AND DISCUSSION

The research results were analysed statistically. The effect of L-ascorbic acid (AA) on saturated, monounsaturated and polyunsaturated fatty acids was evaluated. The results are presented in the figures below. The samples enriched with L-ascorbic acid are marked MK, the samples without L-ascorbic acid – M.

Table 1 and Figure 1 summarise the results of the research on polyunsaturated fatty acids. During storage in the first 7-days, slight changes in the content of all polyunsaturated acids occurred in the samples fortified and non-fortified with ascorbic acid. This could be described as an induction period. Over the next weeks, a drop in the content of those acids (from 3% to 2.8%) could be observed in M samples (non-enriched), while in MK samples their content increased (to 3.3%). In both cases, the changes can be represented with a second degree function. An analysis using the Student's t-test proved that the influence of ascorbic acid addition was significant (in statistical terms at least) only during the third and the fourth week of storage. After 7 and 14 days of storage, the differences between arithmetical mean values were so insignificant

TABLE 1. Changes in the content (%) of total polyunsaturated fatty acids in the tested samples.

Time of storage (weeks)	Without addition of AA			With addition of AA			t
	$\bar{x}$	S_x	V_x	$\bar{x}$	S_x	V_x	
0	3.01	0.15	4.92	3.00	0.14	4.66	0.001
1	2.99	0.08	2.67	2.97	0.17	5.72	0.007
2	2.94	0.03	1.02	3.03	0.10	3.46	0.882
3	2.91	0.11	3.78	3.22	0.08	2.67	2.891*
4	2.77	0.12	4.33	3.30	0.06	1.94	3.305*
Analysis of regression							
R^2	0.945			0.926			

* indicates rejection of zero hypothesis at the level of $\alpha = 0.05$.

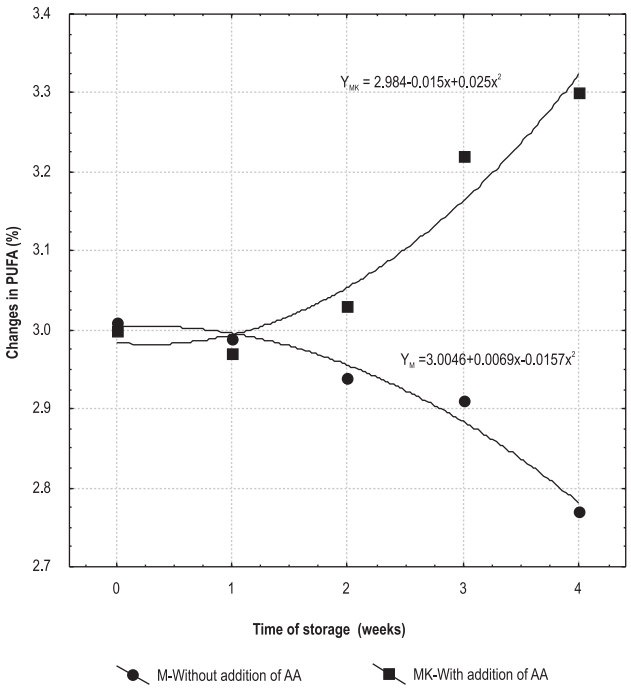

FIGURE 1. Changes in the content of total polyunsaturated fatty acids in butter stored at 4°C.

that "t" test results allowed for adopting the zero hypothesis; however, after three and four weeks the zero hypothesis had to be abandoned. For both periods of storage, the content of all polyunsaturated fatty acids in MK samples was significantly higher than their content in the M samples (the differences are: 0.3 and 0.5%, respectively).

Table 2 and Figure 2 show data on monounsaturated fatty acids. During the storage, systematic changes of the content of all monounsaturated acids occurred in the test samples. These changes happened over the 14-day period at the same rate in both fortified and non-fortified samples (the increase was from approx. 24% up to 24.6–24.7%). In the following weeks a slight decrease in the content of the acids was observed in M samples (up to 24.5%), whereas in the MK samples a further significant increase (up to 25.5%) was taking place. The changes in the test samples could be demonstrated by the second degree function. The analysis

TABLE 2. Changes in the content (%) of total monounsaturated fatty acids in the tested samples.

Time of storage (weeks)	Without addition of AA			With addition of AA			t
	$\bar{x}$	S_x	V_x	$\bar{x}$	S_x	V_x	
0	24.17	0.763	3.02	24.14	1.583	6.55	0.001
1	24.56	1.994	8.18	24.33	2.395	9.84	0.479
2	24.64	0.737	2.99	24.81	0.917	3.75	0.663
3	24.63	0.493	2.00	25.17	0.343	1.37	2.502*
4	24.49	0.603	2.46	25.25	0.520	2.05	3.115*
Analysis of regression							
R^2	0.973			0.927			

* indicates rejection of zero hypothesis at the level $\alpha = 0.05$

TABLE 3. Changes in the content (%) of total saturated fatty acids in the tested samples.

Time of storage (weeks)	Without addition of AA			With addition of AA			t
	$\bar{x}$	S_x	V_x	$\bar{x}$	S_x	V_x	
0	71.82	0.721	1.00	71.88	1.143	1.59	0.002
1	71.61	0.407	0.56	71.15	0.573	0.80	0.043
2	72.05	0.770	1.06	73.03	1.117	1.52	0.088
3	72.19	0.391	0.54	72.06	0.421	0.58	0.019
4	72.28	0.507	0.70	71.67	0.553	0.77	0.145
Analysis of regression							
R^2	0.992			0.988			

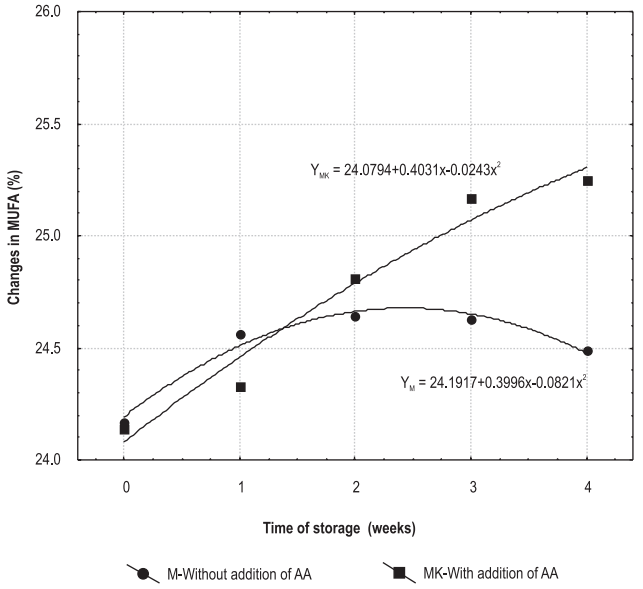

FIGURE 2. Changes in the content of total monounsaturated fatty acids in butter stored at 4°C.

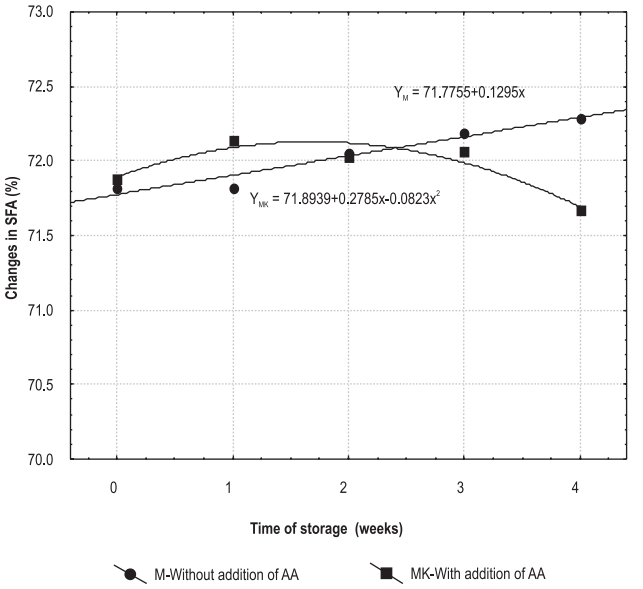

FIGURE 3. Changes in the content of total saturated fatty acids in butter stored at 4°C.

using the Student's t-test showed that the effect of addition of ascorbic acid was significant (in statistical terms at least) only in the third and fourth week of storage of butter. After 7 and 14 days of storage, the differences between arithmetical mean values were so insignificant that "t" test results allowed for accepting the zero hypothesis, though after three and four weeks the zero hypothesis had to be rejected. For both periods of storage, the content of all monounsaturated fatty acids in MK samples was substantially higher than their content in the M samples (the differences were respectively: 0.6 and 0.8%).

Table 3 and Figure 3 show data on saturated fatty acids. During the tests, marginal changes were observed in contents of those acids. In the samples without ascorbic acid (M), a constant increase of this standard occurred (by 0.5% over 4 weeks), while an insignificant increase and then a decrease (the fluctuation of up to 0.5%) were observed in the MK samples. Those changes could be expressed by a second degree function (MK samples) or first degree function (M samples). The analysis with the t-Student method proved that during the whole period of storage the differences between arithmetical mean values were sufficiently low to justify acceptance of the zero hypothesis, *i.e.* the statement that the addition of ascorbic acid did not affect variation in the above-mentioned standard.

CONCLUSIONS

In the test samples, ascorbic acid affected fatty acid contents in different ways. The addition of ascorbic acid affected concentrations of mono- and polyunsaturated fatty acids in a statistically significant manner – the total amount of those acids was higher in fortified samples but only after the third and the fourth week of storage. However, the addition of ascorbic acid did not influence changes in the amount of saturated fatty acids to a statistically significant extent. The studies have shown that variations in the content of unsaturated fatty fatty acids in butter during storage resulting from the addition of L-ascorbic can be described by means of a second-degree mathematical function.

REFERENCES

1. Ayar A., Ozcan M., Akgul A., Akin N., Butter stability as affected by extracts of sage, rosemary and oregano. J. Food Lipids, 2001, 8, 15-25.
2. Hadolin M., Knez Z., Bauman D., Stabilisation of butter with rosemary antioxidants. Acta Alim., 2005, 34, 13-21.
3. International IDF Standard 184:1999. Milk fat. Preparation of fatty acid methyl esters.
4. Jensen R.G., Ferris A.M., Lammi-Keefe C.M., The composition of milk fat. J. Dairy Sci., 1991, 74, 3228-3243.
5. Ozcan M., Ayar A., Effect of propolis extracts on butter stability. J. Food Qual., 2003, 26, 65-73.
6. Pijanowski E., Zarys chemii i technologii mleczarstwa. 1974, vol. II. PWRiL, Warszawa (in Polish).
7. Przybojewska B., Rafalski H., Fatty acids in milk versus human health. Short-chain saturated fatty acids SCFA. Przegl. Mlecz., 2003, 4, 148-151 (in Polish).
8. Staniewski B., Lipolysis in the butter production process. Przem. Spoż., 2000, 7, 34-36, 40 (in Polish).
9. Wefers R., Sies H., The protection by ascorbate and glutatione against microsomal lipid peroxidation is dependent on vitamin E. Eur. J. Biochem., 1988, 174, 353-357.
10. Żegarska Z., Rafałowski R., Amarowicz R., Karamać M., Shahidi F., Stabilization of butter with deodorized rosemary extract. Food Res. Technol., 1998, 206, 99-102.

Received March 2007. Revision received and accepted July 2007.

WPŁYW DODATKU KWASU L-ASKORBINOWEGO NA ZMIANY ZAWARTOŚCI KWASÓW TŁUSZCZOWYCH W MAŚLE PODCZAS PRZECHOWYWANIA

Regina Borek-Wojciechowska

Katedra Nauk o Jakości, Wydział Ekonomiczny, Politechnika Radomska, Radom

Masło nadal należy do tłuszczów chętnie kupowanych przez konsumentów. Bogactwo składników sprawia, że jest to tłuszcz o wyjątkowej wartości odżywczej. Istotnym problemem są zmiany przechowalnicze, szczególnie zmiany jakim ulegają zawarte w maśle kwasy tłuszczowe. Dlatego podejmowane są próby spowolnienia tych zmian. W prezentowanych badaniach podjęto próbę określenia wpływu dodatku kwasu L-askorbinowego na zawartość kwasów tłuszczowych w maśle w trakcie przechowywania w temp. 4°C.

Wyniki badań, poddane analizie statystycznej pozwalają stwierdzić, że dodatek kwasu L-askorbinowego wpływał w sposób statystycznie istotny na zawartość kwasów jedno i wielonienasyconych. Suma tych kwasów była wyższa w próbkach wzbogaconych w kwasy, ale dopiero po upływie 3 i 4 tygodnia przechowywania. Dodatek kwasu L-askorbinowego nie wpłynął natomiast w sposób statystycznie istotny na zmiany zawartości sumy kwasów nasyconych.

Badania wykazały, że zmiany zawartości nienasyconych kwasów tłuszczowych w przechowywanym maśle, wywołane dodatkiem kwasu askorbinowego, można opisać funkcją kwadratową.