POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2006, Vol. 15/56, SI 1, pp. 183–186

COPPER CONTENT OF MILK FAT

Ryszard Rafałowski, Zofia Żegarska

Chair of Commodity Science and Food Research, University of Warmia and Mazury in Olsztyn, Olsztyn

Key words: copper content, milk fat, flameless atomic absorption spectrophotometry

The study was aimed at determining the content of copper in milk fat. Analyses were carried out on fat extracted from non-pasteurised sweet cream obtained from bulk milk. Copper was extracted from fat samples with nitric acid and its content was determined with the method of flameless atomic absorption spectrophotometry.

The results obtained indicate that the content of copper in milk fat ranged from 0.010 to 0.089 mg/kg. The average copper content of fat from the summer feeding accounted for 0.037±0.020 mg/kg, and that of fat from the winter feeding was significantly higher (α=0.05) and reached 0.054±0.021 mg/kg.

INTRODUCTION

Copper belongs to a group of microelements essential in a human diet. It is a constituent of *ca.* 30 enzymes, it participates in the production of haemoglobin and cytochromes [Schuschke, 1997; Milne, 1998]. Copper is a component of, among others, superoxide dismutase which, by transforming superoxide radicals to triplet oxygen, protects cells against their destructive activity [Reiche *et al.*, 1998]. Yet, copper may also be toxic to cells when its concentration exceeds the optimal level [Theophanides & Anastassopoulou, 2002].

Copper is considered as one of the major prooxidants of lipids occurring in food. Especially ions of copper (I) have been found a very active catalyst of oxidation processes [Haase & Dunkley, 1970; Webb *et al.*, 1980]. Copper may display a number of prooxidative activities through, *e.g.*: direct reactions with fat, interactions with secondary products of the oxidation process or degradation of hydroxides produced during oxidation. Irrespective of the mode of action, it always generates free radicals [Paz & Molero, 2000]. Copper is especially active catalyst in the oxidation process of triacylglycerols in the presence of proteins and phospholipids.

The content of native copper in milk ranges from 0.021 to 0.1084 mg/kg milk [Sturup & Buchert, 1996]. It has been demonstrated that 15-20% of native copper occurs in membranes of fat globules [Samuelsson, 1966]. The highest content of that microelement has been reported in milk from the initial period of lactation, then its concentration decreases to a relatively stable level in milk from the middle and final period of lactation [Samuelsson, 1966; Walstra & Jenness, 1984]. In milk and dairy products, copper may also originate from contaminations. It is then a more active catalyst of the oxidation processes than the native copper [Walstra & Jenness, 1984].

The study was aimed at determining the content of copper in fat isolated from non-pasteurised sweet cream of bulk milk obtained from summer and winter feeding of cows.

MATERIAL AND METHODS

Material. The experimental material was fat isolated from non-pasteurised 30% sweet cream sampled in a dairy plant in Olsztyn. Analyses were carried out on 57 samples of milk fat, including 38 samples that originated from the summer feeding (June–October) and 19 samples that originated from the winter feeding of cows (December–April).

Analytical methods. Fat to be analysed was extracted with a modified method of Roese-Gottlieb [Thiele & Timmen, 1969].

To determine the content of copper, the samples of fat were prepared according to the method of De Leonardis *et al.* [2000]. Briefly, 2–3 g of fat were weighed exact to 0.0001 g to a propylene test tube. Next, 1 mL of 10% nitric acid Suprapur (Merck) was added to the tube which was then shaken with a frequency of 40 Hz for 30 s. Next, hermetically sealed tubes were fixed in a water bath at a temperature of 50°C for 2 h. Each 30 min, the tubes were shaken for 30 s. After centrifugation at 3000 rpm for 5 min, 0.5 mL of the bottom aqueous-acidic layer were collected to a flask and supplemented with 7 mL of deionised water. The samples were fixed in propylene cups in an autosampler.

Author's address for correspondence: Ryszard Rafałowski, Chair of Commodity Science and Food Research, University of Warmia and Mazury in Olsztyn, Plac Cieszyński 1, 10-726 Olsztyn-Kortowo, Poland; tel.: (48 89) 523 47 29; e-mail: rych@uwm.edu.pl

Standard solutions were prepared using an oil standard of copper (Merck) at a concentration of 1 mg/g.

The content of copper was assayed with the method of flameless atomic absorption spectrophotometry using a Unicam 939/959 spectrophotometer, under the following conditions: lamp current – 4 mA; wave length – 324.8 nm; gap – 0.5 nm; gas – argon, flow rate – 3 L/min; sample's volume – 20 μL. Programme of a graphite furnace: the first phase: 80°C, 10 s; the second phase: 100°C, 45 s; the third phase: 120°C, 10 s; the fourth phase: 700°C, 20 s; the fifth phase: 2300°C, 5 s; and the sixth phase: 2700°C, 3 s.

Two parallel determinations were carried out for each fat sample and two parallel measurements were made for each determination.

RESULTS AND DISCUSSION

In this study, copper was extracted from fat with 10% nitric acid. This method of extraction enabled avoiding troublesome mineralisation of fat samples. Prior to determining copper content of the samples examined, analyses were carried out for the repeatability and recovery of the method applied. Results of those analyses compiled in Table 1 indicate that this method is characterised by a satisfactory repeatability and accuracy. Relative standard deviation calculated for 10 parallel determinations accounted for 7.54%, whereas the average recovery of the copper added – for 101.03±9.85%.

TABLE 1. Evaluation of the repeatability and accuracy of the method of copper determination in milk fat.

Repeatability			
Number of determinations		10	
Average content of copper $\bar{x}_a$ (mg/kg)		0.057	
Standard deviation s (mg/kg)		0.004	
Relative standard deviation v (%)		7.54	
Accuracy			
Number of determinations	4	4	4
Copper content of a weighed amount of fat[1] (μg)		0.114	
Copper addition (μg)	0.100	0.150	0.200
Determined amount (μg)	0.210	0.279	0.312
Difference between determined and added amount (μg)	0.096	0.165	0.198
Recovery (%)	94.46	110.32	99.00
Average recovery (%)		101.03±9.85	

[1] – a weighed sample of fat – 2.0000±0.0001g

Results obtained in determinations of the concentration of copper in milk fat samples (Table 2) demonstrate that the average content of copper in the samples from the summer feeding reached 0.037±0.020 mg/kg. The lowest content of copper was reported for the fat samples from July and August. The average content of copper in milk fat from the winter feeding accounted for 0.054±0.021 mg/kg, with the highest values noted in the samples from February (0.065±0.019 mg/kg). The available literature provides sparse data on copper content of milk fat. According to Samuelsson [1966], as little as 22% of copper present in milk migrates to sweet cream. The content of copper in butter, as determined by a number of authors, ranges from 0.02 to 0.33 mg/kg [Bul. IDF, 1978].

As compared to the reported study, a lower content of copper, *i.e.* from 0.01 to 0.03 mg/kg, has been reported in anhydrous milk fat [Mrowetz & Klostermeyer, 1973; Koegh & Higgins, 1986]. Reference data indicate that the content of that microelement in milk and dairy products is affected by such technological processes as cooling and heating of milk. As a result of milk storage at a temperature of 5°C for several hours, nearly half the native copper from the membranes of fat globules has been demonstrated to shift to plasma [Walstra & Jenness, 1984], whereas heating of milk to a temperature of 60°C has been reported to increase copper content of cream [Haase & Dunkley, 1970]. Results of copper content of milk fat obtained in the present study are similar to the values reported in butter examined in Poland by Falandysz and Kotecka [1994]. The average content of copper in butter analysed by those authors reached 0.06±0.03 mg/kg.

The statistical analysis of the results obtained demonstrated that the fat from the winter feeding contained slightly but significantly more (α=0.05) copper than the fat originating from the June–October period. The available literature lacks data on the content of copper in milk fat depending on the season. The higher concentration of copper in the winter months could have been due to the higher content of that element in feed of cows in that season. Investigations carried out in Australia [Tulloch, 1964] have shown that butter originating from the Australian summer (February-April) was characterised by a higher content of copper than the butter from October. Still, the author explained that fact by the transfer of copper from dairy equipment in the summer season.

TABLE 2. Content of copper in milk fat in the summer and winter feeding.

Fat from summer feeding (n=38)				Fat from winter feeding (n=19)			
months	range	$\bar{x}_a$	s	months	range	$\bar{x}_a$	s
(mg/kg)				(mg/kg)			
June	0.011–0.045	0.026	0.016	December	0.036–0.085	0.056	0.019
July	0.011–0.048	0.020	0.010	January	0.031–0.050	0.040	0.014
August	0.010–0.025	0.024	0.001	February	0.033–0.089	0.065	0.019
September	0.034–0.077	0.054	0.015	March	0.031–0.073	0.049	0.018
October	0.030–0.063	0.049	0.013	April	0.015–0.062	0.036	0.021
VI–X	0.010–0.077	0.037[a]	0.020	XII–IV	0.015–0.089	0.054[b]	0.021

a, b – statistically significant difference at α=0.05

CONCLUSIONS

1. The study demonstrated that the content of copper in milk fat from non-pasteurised sweet cream obtained from bulk milk ranged from 0.010 to 0.089 mg/kg, *i.e.* 0.045 mg/kg on average.

2. Milk fat originating from the winter feeding was characterised by a considerably higher content of copper compared to the fat from the summer feeding. The average concentration of copper in fat samples accounted for 0.054 mg/kg and 0.037 mg/kg, respectively.

REFERENCES

1. Buliński R., Kot A., Kotulas K., Michniewski J., Szydłowska E., Determinations of cadmium, lead, zinc and copper concentrations in bulk milk from the Lubelskie Province and in powdered milk. Rocz. Inst. Przem. Mlecz., 1978, 20, 3, 61–70 (in Polish).
2. Bulletin IDF. Metal contaminations in milk and milk products. 1978, Doc. 105, 14–24.
3. De Leonardis A., Macciola V., De Felice M., Copper and iron determination in edible vegetable oils by graphite furnace atomic absorption spectrometry after extraction with diluted nitric acid. J. Food Sci. Technol., 2000, 35, 371–375.
4. Falandysz J., Kotecka W., Contents of manganese, copper, zinc and iron in dairy products, children's formula and confectionery. Bromat. Chem. Toksykol., 1994, 27, 77–79 (in Polish).
5. Haase G., Dunkley W.L., Copper in milk and its role in catalyzing development of oxidized flavors. Milchwissenschaft, 1970, 25, 656–661.
6. Koegh M.K., Higgins A.C., Anhydrous milk fat. I. Oxidative stability aspects. Irish J. Food Sci. Technol., 1986, 10, 11–12.
7. Milne D.B., Copper intake and assessment of copper status. Am. J. Clin. Nutr., 1998, 67 (suppl.), 1042S–1045S.
8. Mrowetz G., Klostermeyer H., Bestimmung von Eisen und Kupfer in Milchfett aus einem Aufschluß. Z. Lebensm. Unters.-Forch., 1973, 153, 348–353.
9. Paz I., Molero M., Catalytic effect of solid metals on thermal stability of olive oils. J. Am. Oil. Chem. Soc., 2000, 77, 127–130.
10. Reiche D.W., Lillard D.A., Eitenmiller R.R., 1998, Antioxidants. *in*: Food Lipids: Chemistry, Nutrition, Biotechnology (eds. C.C. Akoh, D.B. Min). Marcel Dekker Inc., New York, pp. 423–448.
11. Samuelsson E.G., The copper content in milk and the distribution of copper to some various phases of milk. Milchwissenschaft, 1966, 21, 335–341.
12. Simsek O., Gultekin R., Oksuz O., Kurultay S., The effect of environmental pollution on the heavy metal content of raw milk. Nahrung, 2000, 44, 360–363.
13. Schuschke D.A., Dietary copper in the physiology of the microcirculation. J. Nutr., 1997, 127, 2274–2281.
14. Sturup S., Buchert A., Direct determination of copper and iodine in milk and milk powder in alkaline solution by flow injection inductively coupled plasma mass spectrometry. Fresenius J. Anal. Chem., 1996; 354, 323–326.
15. Theophanides T., Anastassopoulou J., Copper and carcinogenesis. Oncology Hematology, 2002, 42, 57–64.
16. Thiele D., Timmen H., Phosphatid-Phosphor-Bestimmung in Trochenbutter-Reinfett. Milchwissenschaft, 1969, 26, 332–336.
17. Tulloch P.B., A survey of copper content of butter in western Australia. Australian J. Dairy Tech., 1964, 12, 160–162.
18. Walstra P., Jenness R., Dairy Chemistry and Physics. 1984, Wiley Intersci., New York, pp. 85–87.
19. Webb B.H., Johanson A.H., Alford J.A., Fundamentals of Dairy Chemistry. 1980, AVI, Westport-Connecticut, pp. 125–272.

ZAWARTOŚĆ MIEDZI W TŁUSZCZU MLEKOWYM

Ryszard Rafałowski, Zofia Żegarska

Katedra Towaroznawstwa i Badań Żywności, Uniwersytet Warmińsko-Mazurski w Olsztynie, Olsztyn

Celem niniejszych badań była ocena zawartości miedzi w tłuszczu mlekowym. Badaniom poddano tłuszcz wydzielony metodą ekstrakcyjną z niepasteryzowanej śmietanki otrzymanej z mleka zbiorczego. Miedź ekstrahowano z próbek tłuszczu za pomocą kwasu azotowego, a jej zawartość oznaczano metodą bezpłomieniowej spektrofotometrii atomowej.

Uzyskane wyniki wskazują, że zawartość miedzi w tłuszczu mlekowym mieściła się w zakresie od 0,010 do 0,089 mg/kg. Średnia zawartość miedzi w tłuszczu z okresu żywienia pastwiskowego wynosiła 0,037±0,020 mg/kg, a w tłuszczu z okresu żywienia oborowego była istotnie wyższa (α=0,05) i wynosiła 0,054±0,021 mg/kg.