EXTRACTIVE STRIPPING VOLTAMMETRY AT A GLASSY CARBON PASTE ELECTRODE FOR ANALYSIS OF COW'S MILK AND CREAM

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ABSTRACT

In this paper, a procedure based on extractive accumulation of milk fat globules (MFGs) into a pasting liquid (lipophilic binder) of glassy carbon paste electrode (GCPE) with subsequent electrochemical detection by square-wave voltammetry (SWV) in 0.1 mol L^{-1} Britton-Robinson buffer of pH 4.0 has been tested to find out whether it can be utilized as a simple screening analytical method for cow's milk and cream nutrition control. Since there is assumption that the necessary alkaline hydrolysis of cow's milk and subsequent extraction of lipophilic vitamins into an organic solvent could be avoided, several GCPEs differing in type (atactic polypropylene, paraffin oil, paraffin wax, silicone oil, and vaseline) and content (5, 10, 15, 20, and 25%; w/w) of pasting liquid used were tested as part of complex optimization. The obtained results show that MFGs contain predominantly vitamin A (carotenoids and retinoids), especially all-*trans*-retinol, which could serve as significant marker of the fat content. However, their individual forms were not possible to distinguish due to the considerable anodic peak broadening (overlapping).

Keywords: carbon paste electrode; cow's milk; extraction; milk fortification; nutrition control; voltammetry

INTRODUCTION

In the mammary glands, milk fat globules (MFGs), ranging in size from 0.1 to 15 μm in diameter **(Logan et al., 2014)**, originate as fat droplets composed largely (>98%) of triacylglycerols (TCGs). These fat droplets are evenly emulsified throughout the volume and contain lipophilic (fat-soluble) vitamins dissolved in them **(Heid and Keenan, 2005)**. Losses of naturally occurring lipophilic vitamins are significant after mechanical separating the milk fat (cream) from the raw milk. Obtained skimmed milk is then homogenized that is a process of breaking down the large fat droplets under high pressure so that they stay together and do not separate as cream. To improve the nutritional values, the homogenized milk is usually fortified by extra vitamins (retinyl palmitate and cholecalciferol) and minerals that are not naturally found in milk in significant amounts **(Trinidad et al., 2015)**.

The cow's milk and products made from it are considered as very complex sample matrixes and their analysis is often complicated and time-consuming **(Trenerry et al., 2011)**. Valid reference analytical methods used for lipophilic vitamins determination in foodstuffs in laboratories of the Czech Agriculture and Food Inspection Authority 211/2004 Coll4. utilize a HPLC with UV detection, known as standard: ČSN EN 12823 (vitamin A),

ČSN EN 12821 (vitamin D), ČSN EN 12822 (vitamin E) and ČSN EN 14148 (vitamin K). In addition, a gravimetric method (EN 1211) is used to determine milk fat content.

Evaluation of the lipophilic vitamins content in milk (also dairy produce) has its substantiation, especially in case of human nutrition which deals on provision of essential nutrients in food necessary to support human life and health **(Haug et al., 2007)**. Moreover, analytical methods for simultaneous determination of lipophilic vitamins and their provitamins in milk using microcolumn **(Gomis et al., 2000)**, narrow-bore column **(Blanco et al., 2000)** and two-dimensional liquid chromatography **(Zhang et al., 2015)** have been developed.

Time-consuming sample preparation is the most challenging step in the analysis as it involves several steps (alkaline hydrolysis, liquid-liquid extraction, filtration and evaporation of organic solvent) in which the analytes may be lost **(Trenerry et al., 2011)**. To avoid degradation of analytes, the alkaline hydrolysis shoud be carried out in presence of an antioxidant, under an inert atmosphere, and in absence of light.

A simple semiquantitative method for the determination of vitamin D in skim milk is worth mentioning **(Michlová et al., 2012)** when a sample is diluted with water, ethanol, and an aqueous ammonia solution. Vitamin D is subsequently extracted with a mixture of ether and hexane

for 4 hours. After evaporation of the organic solvent, vitamin D is transferred to the appropriate solvent (usually methanol or acetronitrile). The advantage of this procedure is that the sample does not need to undergo alkaline hydrolysis.

This paper offers a simple screening voltammetric method for monitoring vitamin A content (sum of retinoids and carotenoids) in cow's milk and cream samples without the need for a use complicated sample preparation. Since all lipophilic vitamins are electrochemically active organic compounds that undergo oxidation (vitamins A, D and E) or reduction (vitamin K) electrode reactions (**Lovander et al., 2018**), they can be directly extracted from the milk into a pasting liquid (nonpolar binder) of glassy carbon paste electrode (GCPE). After transferring GCPE into an aqueous working medium (medium−exchange approach), the electrochemical detection of accumulated vitamins can be performed using a pulse voltammetric technique (**Sýs et al., 2019**), namely square-wave voltammetry (SWV).

Scientific hypothesis

In this work, an effort was to find out whether square-wave anodic stripping voltammetry at GCPE can represent a suitable method for rapid determination of vitamin A.

MATERIAL AND METHODOLOGY
Chemicals and reagents

All-*trans*-retinol (≥95%) and ethanol (≥99.5%) were purchased from Sigma-Aldrich. Universal 0.1 mol.L^{-1} Britton-Robinson buffer (BRB), prepared by mixing of appropriate amounts of boric acid, glacial acetic acid, 85% phosphoric acid, and sodium hydroxide all from the aforementioned company, was used in selection of suitable detection medium. BRB was prepared using deionized water (minimum electric resistivity 18.2 MΩ cm, maximum 3 μg L^{-1} of total organic carbon) made in a Milli-Q® ultrapure water system from Merck Millipore (Burlington, USA).

Instrumentation

Voltammetric detection of accumulated lipophilic vitamins into the pasting liquid was performed in a conventional three-electrode arrangement containing always GCPE (working), silver chloride electrode with 3 mol.L^{-1} KCl salt-bridge (reference) and platinum sheet (auxiliary electrode). These electrodes were connected to the potentiostat Autolab PGSTAT101 controlled by software Nova (Version 1.11.0), both from Metrohm (Prague, Česká republika).

Preparation of carbon paste electrode

Glassy carbon powder of type Sigradur G (mixture 5 – 20 μm, HTW Maintingen, Germany) and one of randomly selected pasting liquids were mixed in a ceramic mortar for 15 min to create homogenous glassy carbon paste. The amount of tested pasting liquid differed from 5 to 30% (w/w). The resulting glassy carbon paste was packed into the cavity of the Teflon® piston-driven electrode holder with an end-hole of 3 mm in diameter. It is necessary to mention that the height of column in the cavity must be less than 2 cm due to difficult extrusion of

glassy carbon paste. It is recommended that freshly prepared GCPEs should not be employed in any experiments due to their rather unstable electrochemical behaviour attributed to an incomplete homogenization. Consequently, freshly prepared GCPEs were left at the laboratory conditions for one day. After this self-homogenization process, GCPEs can be used for following voltammetric measurements (**Sýs et al., 2017**).

Methods

Principle of medium-exchange extractive stripping voltammetry is illustarted in Figure 1. The extraction of lipophilic vitamins into the pasting liquid was carried out from 10 mL non-treated milk and cream samples (available in Czech stores) without need to apply a potential in the electrode cell (nonelectrolytic preconcentration), which is an approach known as "open circle procedure". After 10 min, GCPE enriched with analytes was rinsed with a stream of deionized water and immersed together with others electrodes into 0.1 mol.L^{-1} BRB. Final voltammetric detection was performed using square-wave voltammetry at potential range from 0 to +1.4 V, potential step (E_{step}) of 5 mV, potential amplitude (E_{ampl}) of 25 mV and frequency (f) of 50 Hz.
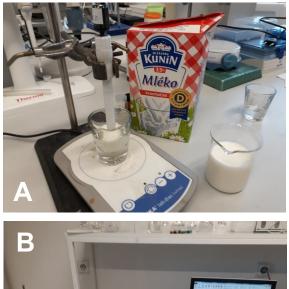

Figure 1 Individual steps of extractive stripping voltammetry with medium-exchange (A; accumulation and B; electrochemical detection using SWV).

Statistic analysis
Extraction repeatability

Generally, the repeability may be expressed by several indexes, nemaly coefficient of repeatability (CR), coefficient of variation (CV) and intra-class correlation

coefficient (ICC). CR defined by formula (1) is a precision measure which represents the value below which the absolute difference between two repeated test results may be expected to lie with a probability of 95%. The standard deviation (σ) under repeatability conditions is part of precision and accuracy.

$$CR = 1.96\sqrt{2\sigma^2} \tag{1}$$

However, the repeatability is more often given by CV defined as the ratio of the standard deviation (σ) and to the mean (μ). If this ratio is expressed as a percentage (see Eq. 2) then it will be referred to as relative standard deviation (RSD).

$$RSD = \frac{\sigma}{\mu} * 100 \tag{2}$$

Sufficient extraction repeatability constitutes the main criterion for development of voltammetric methods utilizing extractive accumulation to be able to use them for analytical purposes. ICC could not be used because units of two physical quantities (variables) were statistically tested only. Therefore, using RSD can be probably expected to be sufficient.

RESULTS AND DISCUSSION
Selection of pasting liquid type
Several GCPEs differing in the type of pasting liquid and containing always 20% (w/w) portion were investigated in SWASV of cow's milk (3.5% fat) to choose the optimum one. Working conditions for this experiment were as follows: accumulation for 10 min, stirring at 400 rpm, electrochemical detection in 0.1 mol.L^{-1} BRB (pH 4.5) at $E_{start} = 0$ V, $E_{end} = +1.4$ V, $E_{step} = 5$ mV, $E_{ampl} = 25$ mV and $f = 10$ Hz. Due to relatively high current response and required reproducibility (Table 1), silicone oil should be taken for optimum extraction of lipophilic vitamins.

Table 1 Comparison of glassy carbon paste electrodes.

Pasting liquid	R (Ω)	E_p (V)	I_p (μA)
Atactic polypropylene	10.5 ±0.8	0.831	0.075
Paraffin oil	7.1 ±0.2	0.851	3.69 ±1.6
Paraffin wax	4.7 ±0.3	0.836	0.24 ±0.1
Silicone oil (8000 cSt)	8.0 ±0.2	0.844	1.14 ±0.2
Vaseline	17.4 ±1.0	0.829	0.013

Note: Values (R; ohmic resistance; E_p; peak potential, I_p; peak current response) given as $\mu \pm 2\sigma$ (95% probability) for five repetitions.

Ratio between carbon powder and pasting liquid
Under the prediction, an amount of extracted lipophilic vitamins would increase with a higher content of paste liquid in GCPE. However, electrochemical properties of GCPE are affected by ratio between glassy carbon powder and paste liquid. In principle, it can be stated that carbon particles remain in intimate contact (electrically conductive) until the amount of paste liquid exceeds 30% (w/w) **(Švancara and Schachl, 1999)**.
Surprisingly, it was found that the highest peak current response (unfortunately, background current (I_b) as well) was obtained at GCPE containing 5% (w/w) silicone oil, as demonstrated in Table 2. Despite high current yield, the

content of 15% (w/w) silicone oil was chosen as optimum, thanks to the high reproducibility of accumulation. A non-specific adsorption of milk fat onto electrode surface, which acts as an electrical insulator causing a significant increase of background current (baseline signal), can be considered as possible explanation.

Table 2 Effect of silicone oil content in GCPE.

Content (%)	R (Ω)	I_p (μA)	I_b (μA)
5	6.7 ±0.2	10.0 ±0.3	66.4 ±3.50
10	7.0 ±0.3	4.7 ±0.3	8.9 ±0.90
15	4.8 ±0.2	5.1 ±0.3	4.4 ±0.50
20	8.0 ±0.2	1.1 ±0.2	0.9 ±0.02
25	6.0 ±0.1	0.9 ±0.1	0.7 ±0.02

Note: Values (R; ohmic resistance; I_p; peak current response; I_b; background current) given as $\mu \pm 2\sigma$ (95% probability) for five repetitions.

Effect of accumulation time
Principally, the optimum value of accumulation time is defined as a period required for reaching the equilibrium of lipophilic vitamins distribution between a nonopolar pasting liquid of GCPE and used milk sample. The cow's milk can be considered as a direct emulsion (so-called the first type emulsion) because a small amount of fat droplets (organic phase) are uniformly distributed throughout the milk volume (aqueous phase).
Resulting saturation curve describing dependence of current peak height on accumulation time showed a typical extraction equilibrium isotherm, as shown in Figure 2. The extraction equilibrium has been achieved after 600 s because using accumulation for longer period did not cause any significant increase in peak current response. Hence, accumulation time of 10 min was chosen as optimum.
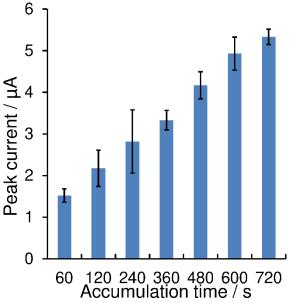

Figure 2 Effect of extraction time on extraction yield of lipophilic vitamins from the cow's milk (3.5% fat).
Note: Data obtained from SWV at GCPE containing 15% (w/w) silicon oil, $E_{step} = 5$ mV, $E_{ampl} = 25$ mV, and $f = 20$ Hz.

Effect of stirring speed

Stirring speed affects the rate of fat droplets transport to the electrode surface where these droples containing lipophilic vitamis are then extracted into the pasting liquid of GCPE. Under this study, it was found that setting the rate of magnetic stir bar higher than 300 rpm did not have any significant effect on increase in final peak current response. Therefore, the above mentioned value can be considered as an optimum for subsequent experiments.

Identification of lipophilic vitamins in cow's milks

The fat dispersed in cow's milks and creams is formed by non-polar TCGs that are surrounded by phospholipids and membrane lipoproteins **(Heid and Keenan, 2005)**. At the natural pH of the cow's milk, they carry a negative charge and thus prevent bonding of MFGs. It is worth considering whether whole MFGs are extracted into the pasting liquid or a further equilibrium distribution of present lipophilic vitamins between the TCGs and the pasting liquid cannot exist. It can be assumed that both processes take place simultaneously. TCGs extracted (adsorbed) block surface of GCPE and therefore cause a dramatic increasing the background current.

From the literature **(Indyk and Woollard, 1997; Hulshof et al., 2006; Trenerry et al., 2011; Musara and Nyagura, 2017)**, lipophilic vitamins are found primarily in the milk fat. Unlike cholecalciferol (vitamin D3) and phylloquinone (vitamin K1) present in limit amounts (0.1 μg per 100 g), α-tocopherol (vitamin E), and retinol together with its provitamines (carotenoids) such as β-carotene, zeaxanthine and luteine (vitamin A) are the major representatives (40-110 μg per 100 g). Moreover, an artificially added retinyl palmitate **(Jensen et al., 1991)** can be present as well.

Generally, most extracted lipophilic vitamins and their provitamins usually provide very broad sensitive oxidation/reduction peaks (up to 250 mV) due to slow kinetic of corresponding electrode reactions occurred at liquid-liquid interface **(Sýs et al., 2019)**. As confirmation, a broad anodic peak beginning +0.705 V at and ending at +1.007 V was obtained for all investigated cow's milks and creams.

It is therefore impossible to distinguish and determine the individual forms of retinoids and carotenoids **(Žabčíková et al., 2018)**. Nevertheless, a number of published scientific papers suggest that the all-*trans*-retinol occupies a dominant position **(Jensen, 1994; Hulshof et al., 2006; Hodulová et al., 2015)**. Thus, it can be assumed that the peak obtained most likely corresponds to the anodic oxidation of all-*trans*-retinol a +0.852 V (compare with (overlapping peak at +0.886 V for cow's milk), as shown in Figure 3.

It seems that proposed extractive stripping voltammetry (ExSV) based on direct immersing of GCPE into continuously stirred cow's milk (3.5% fat) and subsequent electrochemical detection using SWV provides the desired sensitivity for detecting the sum of retinoids and carotenoids. A quantitative or at least semi-quantitative determination of vitamin A in cow's milk and cream samples was not the aim of this study. Voltammetric analysis of cow's milk enriched by differently defined amounts of all-*trans*-retinol could be probably considered as semi-quantitative analytical method.
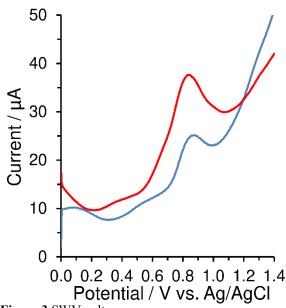

Figure 3 SWV voltammogram.
Note: SWV voltammogram of extracted (at 400 rpm for 10 min) cow's milk (3.5% fat) into GCPE containing always 15% (w/w) silicone oil with subsequent voltammetric detection in 0.1 mol.L^{-1} BRB (pH 4.5) at E_{step} = 5 mV, E_{ampl} = 25 mV, and f = 50 Hz (blue). SWV voltammogram of all-*trans*-retinol extracted (at 400 rpm for 5 min) from its (500 μmol.L^{-1}) 60% ethanolic solutions into GCPE containing always 20% (w/w) silicone oil with subsequent voltammetric detection in 0.1 mol.L^{-1} BRB (pH 4.5) at E_{step} = 1 mV, E_{ampl} = 25 mV, and f = 25 Hz (red line).

Selection of detection medium and supporting electrolyte

Optimisation consisted in finding out proper working conditions for an anodic oxidation of lipophilic vitamins which are presented in MFGs accumulated into silicone oil. At first, the electrochemical detection has been subjected to pH study which was investigated for 0.1 mol.L^{-1} RBRs of pH values from 2 to 7. A linear relationship between peak potential and pH values of used supporting electrolytes, statistically evaluated as E_p = -0.0558 pH + 1.0891 (R^2 = 0.9978), was observed. The peak potential was shifted to more negative values with increased pH of used BRBs. This phenomenon probably occurs due to lowering the energy barrier and easier deprotonation of present lipophilic vitamins. The value of slope 0.0558 indicates the transition of electrons together with protons in a 1:1 ratio. The most sensitive peak current response was achieved using BRBs of pH values 4 and 5. It seems that BRB would be replaced by an acetate buffer of pH 4.5, more simple in composition.

Optimization of square-wave voltammetry

Parameters of SWV, potential amplitude and frequency, were optimised at constant potential step of 5 mV. Extracted lipophilic vitamins provided a broad anodic peak which height increased with increasing potential amplitude up to value of 25 mV. Therefore, potential amplitude of 25 mV was chosen for following analysis of cow's milks and creams. As shown in Figure 4, the height of anodic peak linearly increased with higher frequency. However, it

was observed that background current (baseline) increased as well. Hence, a value of 50 Hz representing a compromise was chosen as optimum.
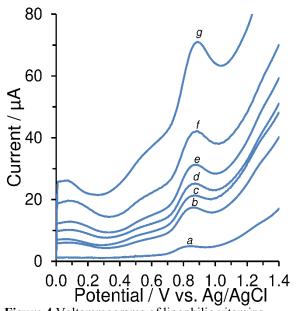

Figure 4 Voltammograms of lipophilic vitamins.
Note: Voltammograms of lipophilic vitamins sum (predominantly all-*trans*-retinol and β-carotene) extracted from cow's milk (3.5% fat) in GCPE containg 15% (w/w) silicone oil at 300 rpm and for 10 min. After rinsing with distilled water, subsequent voltammetric detection was performed in 0.1 mol.L^{-1} BRB (pH 4.5) at E_{step} = 5 mV, E_{ampl} = 25 mV, and f = 10 (*a*), 20 (*b*), 30 (*c*), 40 (*d*), 50 (*e*), 60 (*f*) and 100 (*g*) Hz.

Analysis of cow's milks and creams

At first, it is necessary to mention that a sample of whipped cream (40% fat content) could not be analysed using designed protocol due to high viscosity (**Van Vliet and Walstra, 1980**). The whipped cream completely covered GCPE used and did not allow rinsing the surface with distilled water. At first glance, someone may think that peak current will be higher for creams (*'more milk fat more vitamins'*) (**Gaucheron, 2011**). According to Figure 5, demonstrating a dependence of anodic peak current on milk fat content in selected samples, it seems that the assumption is misleading. An explanation could be summarized as follows: lipophilic vitamins present in samples are equally distributed between pasting liquid and milk fat during (*'liquid-liquid'*) extraction and these vitamins are more detained in creams (12 - 31%) than in milks (0.5 - 3.5%) due to many times higher fat content (*'like dissolves like'*).

Extraction repeatability

Assuming that MFGs are homogeneously dispersed throughout the volume of cow's milks and creams and their diameter is not higher than 1 μm (**Robin and Paquin, 1991; Michalski et al., 2004**), an extraction repeability will be affected only by homogeneity of glassy carbon paste used (**Sýs et al., 2017**). If recovery of developed HPLC-based methods ranging approximately from 85 to 110% (**Blanco et al., 2000; Gomis et al., 2000**) is taken to account, satisfactory repeatability of extraction characterized by RSD lower than 9% was achieved. Even despite the relatively short error bars (see Figure 5), it is not possible to determine, on the basis of the peak heights received, whether it is cream or milk with a 3.5% fat content.
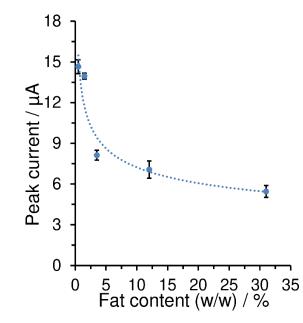

Figure 5 Dependence of peak current response (anodic oxidation of present lipophilic vitamins) on the content of fat in cow's milks and creams.

CONCLUSION

Everyone agrees that the most important part of whole analysis represents a sample preparation at which significant losses of analytes may occur and cannot be detected during the final analysis. Because the extractive stripping voltammetry requires a minimum sample preparation, it was tested as suitable analytical tool for cow's milks and creams quality control. However, the results obtained suggest that direct extraction of lipophilic vitamins (dominantly all-*trans*-retinol) from a continuously stirred sample and subsequent voltammetric detection using square-wave voltammetry could only be used for semi-quantitative determination of milk fat, at this stage of development. Finally, it can be concluded that the scientific hypothesis was refuted.

REFERENCES

Blanco, D., Fernández, M. P., Gutiérrez M. D. 2000. Simultaneous determination of fat-soluble vitamins and provitamins in dairy products by liquid chromatography with a narrow-bore column, *Analyst*, vol. 125, no. 3, p. 427-431. https://doi.org/10.1039/A909027D

Gaucheron, F. 2011. Milk and dairy products: a unique micronutrient combination. *Journal of the American College of Nutrition*, vol. 30, no. S5, p. 400S-409S. https://doi.org/10.1080/07315724.2011.10719983

Gomis, D. B., Fernández, M. P., Gutiérrez M. D. 2000. Simultaneous determination of fat-soluble vitamins and provitamins in milk by microcolumn liquid chromatography, *Journal of Chromatography A*, vol. 891, no. 1, p. 109-114. https://doi.org/10.1016/S0021-9673(00)00623-3

Haug, A., Høstmark, A. T., Harstad, O. M. 2007. Bovine milk in human nutrition – a review, *Lipids in Health and Disease*, vol. 6, no. 25, p. 1-16. https://doi.org/10.1186/1476-511X-6-25

Heid, H. W., Keenan, T. W. 2005. Intracellular origin and secretion of milk fat globules, *European Journal of Cell Biology*, vol. 84, no. 2-3, p. 245-258. https://doi.org/10.1016/j.ejcb.2004.12.002

Hodulová, L., Vorlová, L., Kostrhounová, R., Klimešová-Vyletělová, M., Kuchtík, J. 2015. Interspecies and seasonal differences of retinol in dairy ruminant´s milk. *Potravinarstvo*, vol. 9, no. 1, p. 201-205. http://dx.doi.org/doi:10.5219/436

Hulshof, P. J. M., van Roekel-Jansen, T., van de Bovenkamp P., West, C. E. 2006. Variation in retinol and carotenoid content of milk and milk products in The Netherlands, *Journal of Food Composition and Analysis*, vol. 19, no. 1, p. 67-75. https://doi.org/10.1016/j.jfca.2005.04.005

Indyk, H. E., Woollard, D. C. 1997. Vitamin K in milk and infant formulas: determination and distribution of phylloquinone and menaquinone-4, Analyst, vol. 122, no. 5, p. 465-469. https://doi.org/10.101610.1039/A608221A

Jensen, R. G., Ferris, a. M., Lammi-Keefe, C. J. 1991. The composition of milk fat, *Journal of Dairy Science*, vol. 74, no. 9, p. 3228-3243. https://doi.org/10.3168/jds.S0022-0302(91)78509-3

Jensen, S. K. 1994. Retinol determination in milk by HPLC and fluorescence detection, *Journal of Dairy Research*, vol. 61, no. 2, p. 233-240. https://doi.org/10.1017/S0022029900028247

Logan, A., Auldist, M., Greenwood, J., Day, L. 2014. Natural variation of bovine milk fat globule size within a herd, *Journal of Dairy Science,* vol. 97, no. 7, p. 4072-4082. https://doi.org/10.3168/jds.2014-8010

Lovander, M. D., Lyon, J. D., Parr, D., Wang, J., Parke, B., Leddy, J. 2018 Critical Review—Electrochemical properties of 13 vitamins: a critical review and assessment, *Journal of the Electrochemical Society*, vol. 165, no. 2, p. G18-G49. https://doi.org/10.1149/2.1471714jes

Michalski, M. C., Camier, B., Briard, V., Leconte, N., Gassi, J. Y., Goudédranche, H., Michel, F., Fauquant, J. 2004. The size of native milk fat globules affects physico-chemical and functional properties of Emmental cheese, *Dairy Science and Technology,* vol. 84, no. 4, p. 343-358. https://doi.org/10.1051/lait:2004012

Michlová, T., Hejtmánková, A., Pivec, V., Dragounová, H., Hejtmánková. K., Elich, O. 2012. Vliv pasterace a zamrazení na obsah lipofilních vitaminů v mléce, *Mlékařské listy,* vol. 135, no. 135, p. 5-9.

Musara, C., Nyagura, M. 2017. Skimmed milk as a determinant of vitamin A deficiency, *South African Journal of Clinical Nutrition,* vol. 30, no. 1, 24-26. http://dx.doi.org/10.1080/16070658.2017.1237454

Robin, O., Paquin, P. 1991. Evaluation of the particle size of fat globules in a milk model emulsion by photon correlation spectroscopy, *Journal of Dairy Science,* vol. 74, no. 8, p. 2440-2447. https://doi.org/10.3168/jds.S0022-0302(91)78419-1

Sýs, M., Farag, A. S., Švancara, I. 2019. Extractive stripping voltammetry at carbon paste electrodes for determination of biologically active organic compounds, *Monatshefte für Chemie*, vol. 150, no. 3, p. 373-386. https://doi.org/10.1007/s00706-018-2346-0

Sýs, M., Žabčíková, S., Červenka, L., Vytřas, K. 2017. Comparison of adsorptive with extractive stripping voltammetry in electrochemical determination of retinol, *Potravinarstvo Slovak Journal of Food Sciences,* vol. 11, no. 1, p. 96-105. https://dx.doi.org/10.5219/713

Švancara, I., Schachl, K. 1999. Testing of unmodified carbon paste electrodes, *Chemické Listy*, vol. 93, no. 8, p. 490-499.

Trenerry, V. C., Plozza, T., Caridi, D., Murphy, S. 2011. The determination of vitamin D3 in bovine milk by liquid chromatography mass spectrometry, *Food Chemistry*, vol. 125, no. 4, p. 1314-1319. https://doi.org/10.1016/j.foodchem.2010.09.097

Trinidad, T. P., Mallillin, A. C., Sagum, R. S., de Leon, M. P., Borlagdan, M. S., Baquiran, A. F. P. 2015. Fortified milk consumption among 6-year old children: changes in biochemical markers of trace minerals and vitamins, *Trace Elements and Electrolytes*, vol. 32, no. 3, p. 1-7. https://doi.org/10.5414/TEX01376

Van Vliet, T., Walstra, P. 1980. Relationship between viscosity and fat content of milk and cream. *Journal of Texture Studies,* vol. 11, no. 1, p. 65-68. https://doi.org/10.1111/j.1745-4603.1980.tb00308.x

Zhang, Y., Qibule, H., Jin, Y., Wang, J., Ma, W. 2015. Simultaneous determination of vitamins A, D3 and E in infant formula and adult nutritions by online two-dimensional liquid chromatography, *Chinese journal of chromatography*, vol. 33, no. 3, p. 291-297. https://doi.org/10.3724/SP.J.1123.2014.11009

Žabčíková, S., Mikysek, T., Červenka, L., Sýs, M. 2018. Electrochemical study and determination of all-trans-retinol at carbon paste electrode modified by a surfactant, *Food Technology and Biotechnology*, vol. 56, no. 3, p. 337-343. https://doi.org/10.17113/ftb.56.03.18.5618

Conflict of interest:
All authors declare no conflict of interest.

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