Encapsulation of L-5-methyltetrahydrofolate by electrospraying for food applications

Ložnjak Švarc, Petra; García Moreno, Pedro Jesús; Mendes, Ana Carina Loureiro; Fallahasghari, Elnaz Z.; Jakobsen, Jette

Published in:
Journal of Food Engineering

Link to article, DOI:
[10.1016/j.jfoodeng.2019.109901](https://doi.org/10.1016/j.jfoodeng.2019.109901)

Publication date:
2020

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Ložnjak Švarc, P., García Moreno, P. J., Mendes, A. C. L., Fallahasghari, E. Z., & Jakobsen, J. (2020). Encapsulation of L-5-methyltetrahydrofolate by electrospraying for food applications. *Journal of Food Engineering*, 277, [109901]. <https://doi.org/10.1016/j.jfoodeng.2019.109901>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Journal Pre-proof

Encapsulation of L-5-methyltetrahydrofolate by electrospraying for food applications

Petra Ložnjak Švarc, Pedro J. García-Moreno, Ana C. Mendes, Elnaz Z. Fallahasghari, Jette Jakobsen

PII: S0260-8774(19)30544-8

DOI: <https://doi.org/10.1016/j.jfoodeng.2019.109901>

Reference: JFOE 109901

To appear in: *Journal of Food Engineering*

Received Date: 28 October 2019

Revised Date: 12 December 2019

Accepted Date: 28 December 2019

Please cite this article as: Ložnjak Švarc, P., García-Moreno, P.J., Mendes, A.C., Fallahasghari, E.Z., Jakobsen, J., Encapsulation of L-5-methyltetrahydrofolate by electrospraying for food applications, *Journal of Food Engineering* (2020), doi: <https://doi.org/10.1016/j.jfoodeng.2019.109901>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier Ltd.

Title:

Encapsulation of L-5-methyltetrahydrofolate by electrospraying for food applications

Authors:

Petra LOŽNJAK ŠVARC^{a,*}, Pedro J. GARCÍA-MORENO^{a,b}, Ana C. MENDES^c, Elnaz Z. FALLAHASGHARI^{a,c}, Jette JAKOBSEN^a

^aResearch Group for Bioactives – Analysis and Application, National Food Institute, Technical University of Denmark, Kemitorvet, 2800 Kgs. Lyngby, Denmark

^bDepartment of Chemical Engineering, University of Granada, Avenida de la Fuente Nueva S/N P.C 18071 Granada 18071, Spain

^cResearch Group for Nano-Bio Science, National Food Institute, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

*Corresponding author: Petra Ložnjak Švarc, Research Group for Bioactives – Analysis and Application, National Food Institute, Technical University of Denmark, Kemitorvet, 2800 Kgs. Lyngby, Denmark, Telephone: + 45 93518869, E-mail: petlon@food.dtu.dk

Abstract:

Biologically active natural folate form L-5-methyltetrahydrofolate is less stable than synthetic folate form folic acid commonly used for food fortification. The production of electrosprayed capsules using a combination of carbohydrates such as glucose syrup and pullulan was investigated to provide higher stability of L-5-methyltetrahydrofolate in food products. Additionally, the protective effect of ascorbic acid, if added to the biopolymer solution for capsules production was investigated. During the production of electrosprayed capsules containing L-5-

methylnetetrahydrofolate, both with and without ascorbic acid, the recovery of folate was >97%. During storage at 22°C for 21 days, the electrosprayed capsules (with or without ascorbic acid) showed significantly higher folate recovery compared to free L-5-methylnetetrahydrofolate (91% vs. 61%, $p \leq 0.05$). In buns baked with all-purpose flour fortified with the free form or the electrosprayed capsules, which were stored at 22 °C for 9 days, no significant difference was shown for the retention of the L-5-methylnetetrahydrofolate.

Keywords:

L-5-Methylnetetrahydrofolic acid; Fortification; LC-MS/MS; Food; Stability; Retention

Abbreviations:

NTDs, Neural tube defects; 5-CH₃-H₄folate, 5-methylnetetrahydrofolate, natural form; L-5-CH₃-H₄folate, L-5-methylnetetrahydrofolate, synthetic form; Asc, Ascorbic acid; *Capsules – Asc*, Capsules without ascorbic acid; *Capsules + Asc*, Capsules containing ascorbic acid

1. Introduction

5-methylnetetrahydrofolate (5-CH₃-H₄folate) is the predominant naturally occurring form of folate or vitamin B₉ group. This group of biologically active compounds plays an important role in human health as it is vital for one-carbon transfer and normal cellular functions such as methylation and regeneration of DNA, where 5-CH₃-H₄folate is involved in biologic processes (Scaglione and Panzavolta, 2014). Adequate folate intake prevents the development of neural tube defects (NTDs), anemia, cardiovascular diseases, cancer, and degenerative cognitive diseases (Bailey et al., 2015). Even though naturally occurring folate is widely distributed in food, its intake is often insufficient (Obeid et al., 2016). More than 260,000 pregnancies worldwide are estimated to be affected by NTDs (Garrett and Bailey, 2018), whereas 44% of the pregnancies are unintended, which also

contributes to the birth defects risk as folate plays a significant role in the first months after conception (Bearak et al., 2018). In order to reduce the prevalence of mentioned diseases and to improve the nutritional quality of the food supply, FDA established mandatory fortification of cereal-grain products among the others; flour, bread, macaroni products in the USA and Canada by folic acid, a synthetic and the most stable folate form (Food and Drug Administration, 1996). In the following years, 81 countries mandated fortification of wheat flour, maize flour, or rice by folic acid, which resulted in a significant reduction of NTDs. This led to the conclusion that food fortification by folic acid should be part of most national public health strategies (Garrett and Bailey, 2018). However, folic acid as a synthetic folate form has to pass additional metabolic processes in order to convert to biologically active folate form 5-CH₃-H₄folate. Upon the chronic intake of synthetic form (>200 µg/day), un-metabolized folic acid is circulating in the body, which results in the occurrence of adverse health effects (Stolzenberg-Solomon et al., 2006) and masking of vitamin B₁₂ deficiency (Green et al., 2013). The use of supplements and voluntary fortification of food products with folic acid are recommended in the European Union, but not mandatory (European Union, 2002, 2006). There is a concern about the occurrence of adverse health effects, which presumably resulted in a decrease of voluntary fortification by folic acid in some countries such as Spain (Samaniego-Vaesken et al., 2017). A fortification of 150 µg folic acid per 100 g of food showed to be effective in the reduction of the prevalence of NTDs and the increase in blood folate concentrations in the USA and Canada (Berry et al., 2010).

L-5-CH₃-H₄folate, Metafolin, a synthetic form of natural 5-CH₃-H₄folate is a biologically active folate form that does not have to undergo additional metabolic conversion, it does not mask B₁₂ deficiency as folic acid does and it is more suitable for food fortification (Obeid et al., 2013; Pietrzik et al., 2010). However, L-5-CH₃-H₄folate is very unstable under various environmental conditions such as changes in pH, temperature, oxygen, or light exposure, which makes its

incorporation to food systems challenging (Liu et al., 2012). Green et al. (2013) studied the bioavailability of encapsulated L-5-CH₃-H₄folate and folic acid from fortified wheat rolls and found no differences in bioavailability, indicating that replacing folic acid by encapsulated L-5-CH₃-H₄folate would reduce concerns regarding the masking of B₁₂ deficiency and adverse health effects of chronic consumption of folic acid.

Due to its low stability, the protection of L-5-CH₃-H₄folate in the food system and gastrointestinal tract is desirable. Encapsulation has been proposed as a new technology for packing materials in the form of micro- or nanostructures by entrapping bioactive compounds and protecting them by another substance in order to increase the stability of bioactive compounds during processing and storage (Dias et al., 2015). Spray-drying is the most commonly used encapsulation technique. However, despite the short processing time, spray-drying can cause deterioration of labile ingredients such as folate due to the contact of the droplet/particle with air at high temperature (100-220 °C) in the drying chamber (Jacobsen et al., 2018). Electrospray has shown to be a good alternative to spray-drying since no high temperatures are used during the process (García-Moreno et al., 2018a). Its relevance for the encapsulation of thermally sensitive bioactive compounds is supported by recent research based on the increase of the productivity of the process (Busolo et al., 2019). This technique is based on the use of a high voltage electric field to generate dry polymeric particles. In electrospray, the jet ejected from the Taylor cone breaks down into fine charged droplets from where the solvent evaporates during the flight to a grounder collector, and the result is the formation of nanoparticles (Ghorani and Tucker, 2015). Previous studies examined the encapsulation of L-5-CH₃-H₄folate using coating materials such as pectin, skimmed milk powder, and modified starch during spray-drying (Kitts and Liu, 2015). The recovery, defined as a ratio of concentrations of L-5-CH₃-H₄folate in the final product after the encapsulation and the one initially added to the dry mixture, ranged from 70 to 91%.

92 The properties of biopolymer solutions (e.g., viscosity, surface tension, conductivity), as well as of
93 the solvent (e.g., vapor pressure), highly affect electrospraying process such as stability of Taylor
94 cone and morphology of the capsules (Jacobsen et al., 2018; Tapia-Hernández et al., 2017). Pectin
95 and alginate are often used for encapsulation of folic acid due to the low cost and biocompatibility,
96 even though coating materials such as stearate, skimmed milk powder, and modified starch have
97 also been used (Alborzi et al., 2013; Kitts and Liu, 2015). In this study, glucose syrup was used as
98 the main carbohydrate due to its ability to form capsules of appropriate morphology by
99 electrospraying, whereas pullulan was used to stabilize the electrospraying process (García-Moreno
100 et al., 2018b; Hermund et al., 2019). Glucose syrup is a common and low-cost coating material used
101 for the encapsulation of bioactive compounds for application in food (García-Moreno et al., 2018b).
102 Furthermore, glucose syrup-electrosprayed capsules have lower oxygen permeability when
103 compared to higher molecular weight carbohydrates such as dextran (Boerekamp et al., 2019). The
104 latter is of special importance for the protection of oxidative unstable bioactives such as L-5-CH₃-
105 H₄folate. Besides, the protective effect of ascorbic acid (Asc) against oxidation was also examined,
106 as L-5-CH₃-H₄folate is instable under various environmental conditions (Kitts and Liu, 2015).

107 To the best of our knowledge, the encapsulation of L-5-CH₃-H₄folate by electrospray, that could
108 provide even higher protection of the bioactive compound than spray-drying, remains to be
109 investigated. Electrospraying is performed at room temperature, which is convenient for
110 encapsulation of thermally sensitive compounds such as L-5-CH₃-H₄folate. Furthermore, the
111 reduced capsule size compared to spray-drying enables easier incorporation in a food matrix (Kitts
112 and Liu, 2015). As the main ingredient in bakery products being already used for fortification by
113 folic acid, all-purpose flour seems to be a good candidate to be fortified with 5-CH₃-H₄folate. Due
114 to the costliness of the pure 5-CH₃-H₄folate, the synthetic Metafolin form of L-5-CH₃-H₄folate was
115 used in this study as it was proposed by previous studies (Liu et al., 2015, 2013).

The study aimed to investigate the encapsulation of L-5-CH₃-H₄folate by electrospraying using a combination of carbohydrates, such as glucose syrup and pullulan, as coating materials. The study included optimization of the electrospraying process and testing the oxidative stability of the capsules loaded with L-5-CH₃-H₄folate during 21 days of storage. In addition, the protecting effect of encapsulation during processing was tested by studying the fortification of buns produced with all-purpose flour containing electrosprayed capsules loaded with L-5-CH₃-H₄folate.

2. Materials and methods

2.1. Materials

Glucose syrup (DE38, C*Dry 1934) was kindly provided by Cargill Germany GmbH (Krefeld, Germany). Pullulan (molecular weight = 200,000 Da) and Tween-20 (polyethylene glycol sorbitan monolaurate) were provided by Hayashibara Co., Ltd. (Okayama, Japan) and Sigma-Aldrich (Steinheim, Germany), respectively. L-5-Methyltetrahydrofolic acid (L-5-CH₃-H₄folate, (6S)-5-methyltetrahydrofolic acid, calcium salt, Metafolin) was provided from Merck & Cie (Schaffhausen, Switzerland). All-purpose flour and granulated sugar (Vores bagværk), salt, instant dry yeast (Malteserkors), eggs (Barkholt), and sunflower oil were bought from the local supermarket (Copenhagen, Denmark).

2.2. Optimization of biopolymer solutions for electrospraying process

The main compounds of the electrospraying solution, such as glucose syrup (15%) and pullulan (4%), were selected based on previous work (García-Moreno et al., 2018b). The experiments involved in the optimization of the electrospraying process included the addition of 1% Asc, 1% Tween-20 surfactant, and the use of 3-5 Microfluidizer passes (M110L Microfluidics, Newton, MA, USA). The addition of Asc to the electrospraying solution was carried out to test its effect on the stability of L-5-CH₃-H₄folate in a further study during the electrospraying process, storage, and

processing of the capsules. Tween-20 addition, which reduces the surface tension of the biopolymer solution, and passing the biopolymer solution through a high-pressure homogenizer (e.g., Microfluidizer), which breaks biopolymer chains reducing polymer entanglements, were also carried out in order to evaluate their influence on electrosprayability.

Briefly, pullulan and glucose syrup were dissolved in distilled water under constant stirring (700 rpm) for 30 min at room temperature. Asc was added, dissolved, and further homogenized in a Microfluidizer (M110L Microfluidics, Newton, MA, USA) equipped with a ceramic interaction chamber (CIXC, F20Y, internal dimension 75 μm). The solutions for electrospraying were homogenized at a pressure of 9,000 psi, with 3 passes, whereas 5 passes were also tested. In order to investigate the effect of the surfactant in the process of electrospraying, 1% Tween-20 was added to the solution before and after homogenization. L-5-CH₃-H₄folate was included in the optimization process after the homogenization of the solutions produced in optimal conditions. Solution with L-5-CH₃-H₄folate was stored in a brown glass vial, atmospheric oxygen was substituted by nitrogen, and the vial was slowly turned up and down by hand for 3 min until L-5-CH₃-H₄folate was completely dissolved. The resulting solutions for electrospraying contained 1% wt L-5-CH₃-H₄folate. Two different solutions were tested: one without Asc (*Capsules – Asc*) and one with 1% Asc (*Capsules + Asc*). The biopolymer solutions were prepared up to 3 days before electrospraying and stored at room temperature in brown glass vials. L-5-CH₃-H₄folate was added to the solutions right before the electrospraying process in order to avoid oxidation and possible light degradation. All techniques and analysis including L-5-CH₃-H₄folate were performed under subdued light using brown glass in order to protect photo-sensitive folates.

2.3. Electrospraying process

Electrospraying process was performed at room temperature and environmental conditions of relative humidity (26-50 %). Biopolymer solutions were added to a syringe, which was placed in a syringe pump (New Era Pump Systems, Inc., USA). A 16G needle (Proto Advantage, Canada) was used. A high-voltage electrostatic field was supplied by a high voltage power supply (Gamma High Voltage Research, USA), and applied between the spinneret and a stainless steel 15x15 cm collector plate. The system was set horizontally, where the distance between needle tip and collector was 15 cm. In order to stabilize the Taylor cone and avoid dripping of the solution on the collector, different flow rates and voltages in the ranges of 0.001-0.007 mL/min and 16-20 kV were tested, respectively.

The productivity of the electrospraying process was calculated as the weight (mg) of powder collected in 1 h, and it was used during the optimization of the process.

2.4. Characterization of electrosprayed capsules

2.4.1. Morphology

A Scanning Electron Microscopy (SEM) (Phenom-World B.V., Eindhoven, The Netherlands) was used in order to examine the morphology of the capsules produced by electrospraying. The capsules from different experiments were collected on the foil and kept in a desiccator in order to examine the effect of Asc and surfactant addition, as well as the use of different Microfluidizer passes. Approximately 0.5cm² of aluminum sheet covered with a thin layer of electrosprayed capsules was placed on carbon tape and sputter-coated with gold, 8s, 40 mA utilizing a Q150T Quorum Coater (Quorum Technologies Ltd, East Sussex, UK). The capsule diameter distribution was determined from the micrographs by using an image processing program ImageJ (National Institutes of Health). Size distributions were obtained from a minimum of 100 measurements.

2.4.2. Oxidative stability of L-5-CH₃-H₄folate

Immediately after the electrospraying, a part of the capsules was stored in a desiccator in darkness at room temperature, and the other part at -80 °C. The content of L-5-CH₃-H₄folate was quantified the day the capsules were produced and after a 1-month storage at -80 °C. Furthermore, 1% L-5-CH₃-H₄folate in water solution was prepared and used as a control representing non-encapsulated folate. It was stored at room temperature (22 °C) in darkness for 21 days together with fresh *Capsules + Asc* and *Capsules – Asc*, to simulate storage conditions of cereal-grain products that are commonly stored at room temperature. Vials (4 mL) contained approximately 1 mL of control solution and 150 mg of capsules in order to maintain a similar headspace. At days 0, 3, 7, 14, and 21, three subsamples of each sample were analyzed for the content of L-5-CH₃-H₄folate.

2.5. Stability study in fortified buns

2.5.1. Flour fortification

L-5-CH₃-H₄folate was added, in a free form, or the encapsulated forms (*Capsules – Asc*, and *Capsules + Asc*) to a small amount of flour. This small amount of the mixture was dispersed using mortar and pestle before being mixed with the rest of the flour by a commercial-grade mixer. The final concentration of L-5-CH₃-H₄folate was aimed to be ~150 µg/100 g of flour. Six subsamples of each of the fortified flour were sampled for quantification of L-5-CH₃-H₄folate content, in order to ensure even distribution. Unfortified flour was also analyzed for the folate content (n=3) and was used for the preparation of buns, which was necessary for the calculation of folate retention.

2.5.2. Buns production

12 g of active dry yeast was mixed with 20 g of sugar and 100 mL of water. Yeast was mixed with one egg, 40 g of oil and 400 g of flour was added with 1 tsp salt. The elastic dough was kneaded and separated into 15 balls, from which 12 were placed on a baking tray as presented in Supplementary online material (SOM, Fig. S1), and 3 were analyzed as the dough. Buns were

baked at 200°C for 7.5 min until they were risen and golden brown. Three baking batches were carried out, representing three replicates of processing conditions, where four buns were baked in each batch. They were separated after baking to represent different storage conditions, as it is shown on Fig. 1. The buns were weighed before and after the processing, and folate content was analyzed before and after the thermal procedure.

Figure 1 The design of the storage experiment in buns. It included retention study during baking (1) and stability study during storage for 0, 3, 6, and 9 days (2). Three replicates were sampled at each sampling point. *indicate dough samples sampled for the calculation of the retention (n=3).

2.6. Quantification of 5-CH₃-H₄folate

The quantification of 5-CH₃-H₄folate was performed with a slightly modified version of the LC-MS/MS method described in detail elsewhere (Ložnjak et al., 2019). Instead of 1 g of samples, 0.01 g of capsules were dissolved in 25 mL of folate extraction buffer (50 mM phosphate buffer, 1% Asc, 0.1% β-mercaptoethanol, pH 6.0), and diluted to the concentration of approximately 500 ng/mL. When fortified flour or buns were analyzed, 0.5 g of the sample was mixed with 10 mL of folate extraction buffer. ¹³C₅-5-CH₃-H₄folate internal standard was added for quantification. Samples were shaken vigorously after 15 min and cleaned by strong anion exchange solid-phase extraction prior to detection and quantification by LC-MS/MS (Ložnjak et al., 2019).

2.7. Recovery and retention of 5-CH₃-H₄folate

Calculations of recovery and retention of L-5-CH₃-H₄folate was exemplified by 5-CH₃-H₄folate in Eq. 1 and 2. The recovery of 5-CH₃-H₄folate in capsules was calculated according to Eq. 1:

$$Recovery (\%) = \frac{\text{concentration of 5-CH}_3\text{-H}_4\text{folate}_{\text{quantified}}}{\text{concentration of 5-CH}_3\text{-H}_4\text{folate}_{\text{added}}} * 100 \quad [1]$$

229 The retention of 5-CH₃-H₄folate in buns was calculated according to Eq. 2:

$$230 \quad \text{Retention (\%)} = \left(\frac{\mu\text{g 5-CH}_3\text{-H}_4\text{folate per 100 g of buns} * \text{amount of buns}}{\mu\text{g 5-CH}_3\text{-H}_4\text{folate per 100 g of dough} * \text{amount of dough}} \right) * 100 \quad [2]$$

231 **2.8. Statistical analysis**

232 All results from the stability study of L-5-CH₃-H₄folate in capsules and buns were presented as
 233 means of three independent experiments, i.e., buns were prepared in three batches. One-way
 234 ANOVA was used to test the agreement in the recovery of L-5-CH₃-H₄folate between various
 235 samples within the day, and between different treatments. Tukey-Kramer test was used to examine
 236 the differences between the recovery of L-5-CH₃-H₄folate in the samples with free and two different
 237 types of encapsulated folate in capsules and buns. A p-value ≤ 0.05 was classified as a significant
 238 difference. Statistical analyses were performed using JMP[®] Statistical Discovery software version
 239 13.0 (SAS Institute Inc. Cary, NC, USA). The results were given as a mean $\pm$ standard deviation
 240 (SD).

241 **3. Results and discussion**

242 **3.1. Optimization of biopolymer solutions and electrospraying process**

243 Processing conditions leading to stable Taylor cone, while maintaining high productivity, were
 244 needed to be investigated for the new biopolymer solutions with folate and Asc. Table 1 presents
 245 the overview of the experiments performed during the optimization of the electrospraying process
 246 showing the productivity from each experiment. The processing conditions and biopolymer solution
 247 used in former studies, containing 15% glucose syrup and 4% pullulan, were optimized for
 248 encapsulation of fish oil (20% load) (García-Moreno et al., 2018b). The recommendation for dietary
 249 intake of folate is 300 $\mu\text{g/day}$ (NNR, 2014). Therefore, a low amount of folate vitamer L-5-CH₃-
 250 H₄folate should be added in a biopolymer solution, which in contrast to the addition of

macronutrients should not affect the electrospraying process. Pullulan, which has excellent spinnability properties (García-Moreno et al., 2018b), was added to enhance the stability of the Taylor cone during electrospraying at increased flow rates. This increases the productivity of the electrospraying process, which is required when producing encapsulates for food applications.

Table 1 Chronological overview of the experiments used in optimization of electrospraying process and the results for the productivity

During the optimization, the voltage and flow rate parameters were changed from the ones used by García-Moreno et al. (2018b), because of the instability of the process and formation of droplets during electrospraying. Even though previously optimized conditions (17 kV and 0.007 mL/min) provided the highest productivity (Exp. 3 and 5), reduced flow rate (0.006 mL/min) and increased voltage (20 kV) enabled a stable electrospraying process without dripping and droplets in the collector. It should be noted that, despite the considerable difference in bioactive load (20% fish oil vs. 1% folate), the optimum processing conditions slightly varied. This indicates that the electrosprayability of the solutions tested is mainly controlled by the concentration and molecular weight of the biopolymers used.

Table 1 also shows that the addition of Tween-20 enhanced productivity, as observed when comparing Exp. 2 and 3, or 4 and 5. Tween-20 reduced the surface tension of the biopolymer solution, which implies the use of low voltages to eject the jet from the Taylor cone, leading to a more stable electrospraying process (e.g., less dripping) (García-Moreno et al., 2017). As expected, the addition of Asc also led to higher productivity due to an increase in the solid content (Exp. 8 and 9 in Table 1). Finally, increasing the number of Microfluidizer passes from 3 to 5, which could imply more severe breaking of polymer chains, and thus less polymer chain entanglements did have a clear effect on productivity. For instance, productivity was increased for solutions without Asc

when increasing the number of passes (exp. 7 and 9), whereas the opposite was observed for solutions with Asc (Exp. 8 and 10) (Table 1). New optimized conditions used for the production of capsules are shown in Fig. 2.

Figure 2 Scheme of the optimized electrospraying setup and conditions used in this study

3.2. Morphology and distribution of the capsules

Fig. 3 shows the morphology and particle size distribution of produced capsules. The overall capsule sizes ranged from $0.72 \pm 0.41 \mu\text{m}$ for capsules without Asc to $0.55 \pm 0.34 \mu\text{m}$ for capsules with Asc, which is in accordance to the previous study using the same biopolymers where 60-70% of the capsules were below $1 \mu\text{m}$ (García-Moreno et al., 2018b). Particles evaluated after production had a small particle size and smooth and round shape (Fig. 3), enhanced with the addition of the surfactant Tween-20 (SOM, Fig. S2). Minor fibrils also occurred during electrospraying. Thus, an increased number of Microfluidizer passes (5x) was tested in order to break polymer chain entanglements and reduce fibrils formation. However, a minor occurrence of fibrils was still visible (SOM, Fig. S3), which suggests that no further disruption of biopolymer chains (e.g., pullulan) was carried out after 3 passes. The capsules produced by electrospraying had smaller sizes compared to spray-drying ($10\text{-}50 \mu\text{m}$), which enables better incorporation and more homogeneous distribution in a food product (Kitts and Liu, 2015).

Figure 3 SEM images and particle size distribution of electrosprayed capsules containing 1% Tween-20 surfactant after 3 Microfluidizer passes: (a) Capsules – Asc, (b) Capsules + Asc produced at optimum processing conditions (flow rate 0.006 mL/min , voltage 20 kV)

3.3. Stability of L-5-CH₃-H₄folate-loaded electrosprayed capsules

Recovery of L-5-CH₃-H₄folate from electrosprayed capsules was $98 \pm 6\%$ (n=5) and $97 \pm 5\%$ (n=5) in the *Capsules – Asc* and *+ Asc*, respectively. Recovery was calculated as the mean from 5 measurements from two separated batches for each biopolymer solution. Batch 1 was used for the stability study of the capsules, whereas batch 2 was used for the fortification of flour. Stability of produced capsules was tested during storage in the freezer (-80 °C), which indicated that keeping the capsules in the freezer does not affect the stability of capsules (e.g., morphological changes and recovery of L-5-CH₃-H₄folate) (data not shown). Fig. 4 shows the oxidative stability of L-5-CH₃-H₄folate in capsules during storage at room temperature in darkness. The use of Asc in the capsules did not significantly improve the recovery of L-5-CH₃-H₄folate. However, *Capsules + Asc* showed higher recovery between the days, as *Capsules – Asc* differed significantly between the days. From day 7, recovery of free L-5-CH₃-H₄folate was significantly lower ($p \leq 0.05$) than the recovery of encapsulated L-5-CH₃-H₄folate, and an almost linear decrease was observed within the 21 days.

Figure 4 Recovery (%) of 5-CH₃-H₄folate, free and in electrosprayed capsules during 21 days storage in darkness at room temperature (22°C). Different letters *abc* indicate significant differences between different samples within a day, whereas *uxyz* indicate significant differences within the samples analyzed on different days ($p \leq 0.05$).

3.4. Stability of buns enriched with L-5-CH₃-H₄folate

The distribution of L-5-CH₃-H₄folate, free form, and capsules with or without Asc in flour is shown in Table 2. Blending the free L-5-CH₃-H₄folate into the flour resulted in a more uniform distribution, whereas the distribution in *Capsules – Asc* varied as indicated by high SD. Furthermore, the concentration of measured 5-CH₃-H₄folate in *Capsules + Asc* was significantly lower ($p \leq 0.05$) than in the other two fortified flours. These results indicate that some oxidation

during blending may have occurred and that it is possible that using mortar and pestle during the integration of capsules into flour destroyed some capsules.

Table 2 Distribution of free and encapsulated L-5-CH₃-H₄folate in flour prior to dough preparation

Figure 5 Retention (%) of 5-CH₃-H₄folate from buns enriched with L-5-CH₃-H₄folate, free or in capsules, during 9 days storage at room temperature (22°C) in darkness. Non-enriched buns represent 5-CH₃-H₄folate naturally occurring from the other ingredients in buns, whereas the other samples have added L-5-CH₃-H₄folate. Different letters *abc* indicate significant differences between different samples within the day, whereas *xy* indicate significant difference within the same sample between different days ($p \leq 0.05$).

Fig. 5 presents the retention of L-5-CH₃-H₄folate in enriched buns during 9 days of storage at room temperature (22 °C). No significant difference was found in the control and the buns containing *Capsules + Asc* over 9 days of storage. The recovery of L-5-CH₃-H₄folate in *Capsules – Asc* was significantly different from 100%, and it was equal to $93 \pm 0.3\%$ after production, which indicates that part of L-5-CH₃-H₄folate oxidizes during the production. Electrospraying is a suitable substitute for the spray-drying method in terms of working with thermally sensitive compounds like folate. In contrast to our results, in spray-drying sodium ascorbate have shown a protective effect on the stability of L-5-CH₃-H₄folate in capsules using modified starch as a coating material by providing >95% recovery during the production of the capsules (Kitts and Liu, 2015; Liu et al., 2013).

In general, the use of electrosprayed capsules did not show any protective effect on the L-5-CH₃-H₄folate when incorporated into buns. Many different research works have evaluated the potential of microencapsulation for the protection of food bioactives. However, data about their incorporation into food systems are scarce, even though they are important for the evaluation of capsules'

behavior under processing conditions. Encapsulation by electrospraying has already been proven to stabilize bioactive ingredients, but just a few studies tested the effect of capsules incorporation into a food matrix and how food processing influences the stability of the fortified product (Gómez-Mascaraque et al., 2017; Hermund et al., 2019; Miguel et al., 2019). Liu et al. (2013) encapsulated L-5-CH₃-H₄folate by spray-drying and studied storage stability for 7 days. >50% loss of free L-5-CH₃-H₄folate was reported after baking, and degradation pattern in the recovery of L-5-CH₃-H₄folate was observed in pilot baking bread study between the days 3 and 7. Our results showed high retention (100%) of free L-5-CH₃-H₄folate, which was significantly higher than the retention of the encapsulated form. The same observation was made by Kitts & Liu (2015), who used long-chain fatty acid stearate derived from soybean oil for coating material in encapsulation by spray-drying. Both encapsulated L-5-CH₃-H₄folate and folic acid showed lower recovery than corresponding free forms, which was explained by exceeded melting points of stearate and destruction of the capsules that caused oxidation and degradation. Furthermore, Liu et al. (2012) reported a protective effect of skim milk powder and soy milk powder if added to a solution of L-5-CH₃-H₄folate, which indicates that some antioxidative properties of the food matrix may occur. In non-enriched buns, the retention of 5-CH₃-H₄folate was 50% following baking at 200°C for 7.5 minutes, evidencing the instability of the naturally occurring 5-CH₃-H₄folate. Baked buns were stored for 9 days, and no significant difference in 5-CH₃-H₄folate content was observed during this period, which was considered as a limit after which they would not be consumed. The retention of free L-5-CH₃-H₄folate added to buns significantly decreased from 100% to 88% ($p \leq 0.05$), whereas no significant difference was observed in the retention of encapsulated L-5-CH₃-H₄folate after 9 days of storage. However, a significant difference between retention of L-5-CH₃-H₄folate in two types of capsules was observed between days 0 and 3, which could be connected to reduced uniformity of the capsule dispersion in the flour in *Capsules – Asc* as shown in Table 2. As the

stability of free L-5-CH₃-H₄folate started to decrease, there was no significant difference ($p \leq 0.05$) between the stability of buns containing free L-5-CH₃-H₄folate and capsules on day 9. The decrease in retention of free L-5-CH₃-H₄folate in buns followed the pattern similar to the one observed in the recovery of free L-5-CH₃-H₄folate that was equal to 88% after 7 days of storage (Fig. 4).

Even though electrospraying enabled higher recovery than spray-drying (98% vs. 91%), which supports the hypothesis that thermally labile compounds such as folate may be preserved better than if using spray-drying, no protective effect from encapsulation was observed during buns fortification. Further investigation in alternative food products with a longer shelf life is recommended in order to examine if these electrosprayed capsules would improve the stability of folate vitamers during processing. However, our results were in accordance with Gómez-Mascaraque et al. (2017), who found no significant effect of electrosprayed encapsulates loaded with green tea extract when incorporated into biscuits. These findings emphasize the importance of the further step of incorporation of developed capsules to a real food system as their performance varies depending on numerous parameters, such as physical properties of fortified products, as well as production and processing conditions.

4. Conclusions

High oxidative stability of electrosprayed capsules loaded with L-5-CH₃-H₄folate was obtained within 21 days, as no significant difference was observed on L-5-CH₃-H₄folate recovery during storage, whereas a significant decrease of 40% was reported for the free L-5-CH₃-H₄folate. The use of 1% ascorbic acid as an antioxidant did not show any effect on the stability of the produced capsules. Buns were baked with the capsules and stored for 9 days, which showed no significant difference in retention between enrichment with free and encapsulated L-5-CH₃-H₄folate. However, non-enriched buns containing naturally occurring folate from buns' ingredients showed

significantly lower retention of folates (>50%) during processing. Results showed that food processing is a critical step in the preservation of bioactive compounds even when a protection technique such as encapsulation by electrospraying is applied. Therefore, the study may serve as a basis for further research.

Declarations of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

Acknowledgments

This research was supported by the Technical University of Denmark and by Danish Veterinary and Food Administration.

References

- Alborzi, S., Lim, L.-T., Kakuda, Y., 2013. Encapsulation of folic acid and its stability in sodium alginate-pectin-poly(ethylene oxide) electrospun fibres. *J. Microencapsul.* 30, 64–71.
<https://doi.org/10.3109/02652048.2012.696153>
- Bailey, L.B., Stover, P.J., McNulty, H., Fenech, M.F., Gregory, J.F., Mills, J.L., Pfeiffer, C.M., Fazili, Z., Zhang, M., Ueland, P.M., Molloy, A.M., Caudill, M.A., Shane, B., Berry, R.J., Bailey, R.L., Hausman, D.B., Raghavan, R., Raiten, D.J., 2015. Biomarkers of Nutrition for Development—Folate Review 1–5. *J. Nutr.* 145, 1636–1680.
<https://doi.org/10.3945/jn.114.206599>
- Bearak, J., Popinchalk, A., Alkema, L., Sedgh, G., 2018. Global, regional, and subregional trends in unintended pregnancy and its outcomes from 1990 to 2014: estimates from a Bayesian

- 408 hierarchical model. *Lancet Glob. Heal.* 6, e380–e389. <https://doi.org/10.1016/S2214->
409 109X(18)30029-9
- 410 Berry, R.J., Bailey, L., Mulinare, J., Bower, C., Dary, O., 2010. Fortification of flour with folic
411 acid. *Food Nutr. Bull.* 31, S22–S35. <https://doi.org/10.1136/bmj.323.7323.1198>
- 412 Boerekamp, D.M.W., Andersen, M.L., Jacobsen, C., Chronakis, I.S., García-Moreno, P.J., 2019.
413 Oxygen permeability and oxidative stability of fish oil-loaded electrosprayed capsules
414 measured by Electron Spin Resonance: Effect of dextran and glucose syrup as main
415 encapsulating materials. *Food Chem.* 287, 287–294.
416 <https://doi.org/10.1016/j.foodchem.2019.02.096>
- 417 Busolo, M.A., Torres-Giner, S., Prieto, C., Lagaron, J.M., 2019. Electrospraying assisted by
418 pressurized gas as an innovative high-throughput process for the microencapsulation and
419 stabilization of docosahexaenoic acid-enriched fish oil in zein prolamine. *Innov. Food Sci.*
420 *Emerg. Technol.* 51, 12–19. <https://doi.org/10.1016/j.ifset.2018.04.007>
- 421 Dias, M.I., Ferreira, I.C.F.R., Barreiro, M.F., 2015. Microencapsulation of bioactives for food
422 applications. *Food Funct.* 6, 1035–1052. <https://doi.org/10.1039/c4fo01175a>
- 423 European Union, 2002. Directive 2002/46/EC of the European Parliament and of the Council of 10
424 June 2002 on the approximation of the laws of the Member States relating to food
425 supplements. *Off. J. Eur. Communities* 183, 51–57.
426 <https://doi.org/10.1016/j.jclepro.2010.02.014>
- 427 European Union, 2006. Regulation (EC) No 1925/2006 of the European Parliament and of the
428 Council of 20 December 2006 on the addition of vitamins and minerals and of certain other
429 substances to foods. *Off. J. Eur. Union* L404, 26–38.

- 430 Food and Drug Administration, 1996. Food standards: Amendment of standards of identity for
431 enriched grain products to require addition of folic acid. Fed. Regist. 61, 8781–8797.
- 432 García-Moreno, Pedro J, Mendes, A.C., Jacobsen, C., Chronakis, I.S., 2018a. Biopolymers for the
433 Nano-microencapsulation of Bioactive Ingredients by Electrohydrodynamic Processing, in:
434 Gutiérrez, T.J. (Ed.), Polymers for Food Applications. Springer International Publishing,
435 Cham, pp. 447–479. https://doi.org/10.1007/978-3-319-94625-2_17
- 436 García-Moreno, P.J., Özdemir, N., Stephansen, K., Mateiu, R. V., Echegoyen, Y., Lagaron, J.M.,
437 Chronakis, I.S., Jacobsen, C., 2017. Development of carbohydrate-based nano-microstructures
438 loaded with fish oil by using electrohydrodynamic processing. Food Hydrocoll. 69, 273–285.
439 <https://doi.org/10.1016/j.foodhyd.2017.02.013>
- 440 García-Moreno, Pedro J., Pelayo, A., Yu, S., Busolo, M., Lagaron, J.M., Chronakis, I.S., Jacobsen,
441 C., 2018b. Physicochemical characterization and oxidative stability of fish oil-loaded
442 electrosprayed capsules: Combined use of whey protein and carbohydrates as wall materials. J.
443 Food Eng. 231, 42–53. <https://doi.org/10.1016/j.jfoodeng.2018.03.005>
- 444 Garrett, G.S., Bailey, L.B., 2018. A public health approach for preventing neural tube defects: folic
445 acid fortification and beyond. Ann. N. Y. Acad. Sci. 1414, 47–58.
446 <https://doi.org/10.1111/nyas.13579>
- 447 Ghorani, B., Tucker, N., 2015. Fundamentals of electrospinning as a novel delivery vehicle for
448 bioactive compounds in food nanotechnology. Food Hydrocoll. 51, 227–240.
449 <https://doi.org/10.1016/j.foodhyd.2015.05.024>
- 450 Gómez-Mascaraque, L.G., Hernández-Rojas, M., Tarancón, P., Tenon, M., Feuillère, N., Vélez
451 Ruiz, J.F., Fiszman, S., López-Rubio, A., 2017. Impact of microencapsulation within

- 452 electrosprayed proteins on the formulation of green tea extract-enriched biscuits. *LWT - Food*
 453 *Sci. Technol.* 81, 77–86. <https://doi.org/10.1016/j.lwt.2017.03.041>
- 454 Green, T.J., Liu, Y., Dadgar, S., Li, W., Böhni, R., Kitts, D.D., 2013. Wheat rolls fortified with
 455 microencapsulated L-5-methyltetrahydrofolic acid or equimolar folic acid increase blood folate
 456 concentrations to a similar extent in healthy men and women. *J. Nutr.* 143, 867–871.
 457 <https://doi.org/10.3945/jn.113.174268>
- 458 Hermund, D., Jacobsen, C., Chronakis, I.S., Pelayo, A., Yu, S., Busolo, M., Lagaron, J.M., Rosa, J.,
 459 Akoh, C.C., García-moreno, P.J., 2019. Stabilization of fish oil-loaded electrosprayed capsules
 460 with seaweed and commercial natural antioxidants : effect on the oxidative stability of capsule-
 461 enriched mayonnaise. *Eur. J. Lipid Sci. Technol.* 1–10. <https://doi.org/10.1002/ejlt.201800396>
- 462 Jacobsen, C., García-Moreno, P.J., Mendes, A.C., Mateiu, R. V., Chronakis, I.S., 2018. Use of
 463 electrohydrodynamic processing for encapsulation of sensitive bioactive compounds and
 464 applications in food. *Annu. Rev. Food Sci. Technol.* 9, 525–549.
 465 <https://doi.org/10.1146/annurev-food-030117-012348>
- 466 Kitts, D.D., Liu, Y., 2015. Encapsulation strategies to stabilize a natural folate, L-5-
 467 methyltetrahydrofolic acid, for food fortification practices. *Nanotechnol. Funct. Foods Eff.*
 468 *Deliv. Bioact. Ingredients* 142–157. <https://doi.org/10.1002/9781118462157.ch9>
- 469 Liu, Y., Green, T.J., Kitts, D.D., 2015. Stability of microencapsulated L-5-methyltetrahydrofolate in
 470 fortified noodles. *Food Chem.* 171, 206–211. <https://doi.org/10.1016/j.foodchem.2014.08.129>
- 471 Liu, Y., Green, T.J., Wong, P., Kitts, D.D., 2013. Microencapsulation of L-5-methyltetrahydrofolic
 472 acid with ascorbate improves stability in baked bread products. *J. Agric. Food Chem.* 61, 247-
 473 254. <https://doi.org/10.1021/jf304229b>

- 474 Liu, Y., Tomiuk, S., Rozoy, E., Simard, S., Bazinet, L., Green, T., Kitts, D.D., 2012. Thermal
475 oxidation studies on reduced folate, L-5-methyltetrahydrofolic acid (L-5-MTHF) and strategies
476 for stabilization using food matrices. *J. Food Sci.* 77, 236–243. [https://doi.org/10.1111/j.1750-](https://doi.org/10.1111/j.1750-3841.2011.02561.x)
477 3841.2011.02561.x
- 478 Ložnjak, P., García-Salinas, C., Díaz de la Garza, R.I., Bysted, A., Jakobsen, J., 2019. The use of a
479 plant enzyme for rapid and sensitive analysis of naturally-occurring folates in food by liquid
480 chromatography-tandem mass spectrometry. *J. Chromatogr. A* 1594, 34–44.
481 <https://doi.org/10.1016/j.chroma.2019.02.037>
- 482 Miguel, G.A., Jacobsen, C., Prieto, C., Kempen, P.J., Lagaron, J.M., Chronakis, I.S., García-
483 Moreno, P.J., 2019. Oxidative stability and physical properties of mayonnaise fortified with
484 zein electrosprayed capsules loaded with fish oil. *J. Food Eng.* 263, 348–358.
485 <https://doi.org/10.1016/j.jfoodeng.2019.07.019>
- 486 NNR, 2014. Nordic Nutrition Recommendations. Nord. Nutr. Recomm. 2012. Nord. Counc. Minist.
487 <https://doi.org/10.6027/Nord2014-002>
- 488 Obeid, R., Holzgreve, W., Pietrzik, K., 2013. Is 5-methyltetrahydrofolate an alternative to folic acid
489 for the prevention of neural tube defects? *J. Perinat. Med.* 41, 469–483.
490 <https://doi.org/10.1515/jpm-2012-0256>
- 491 Obeid, R., Oexle, K., Reißmann, A., Pietrzik, K., Koletzko, B., 2016. Folate status and health:
492 Challenges and opportunities. *J. Perinat. Med.* 44, 261–268. [https://doi.org/10.1515/jpm-2014-](https://doi.org/10.1515/jpm-2014-0346)
493 0346
- 494 Pietrzik, K., Bailey, L., Shane, B., 2010. Folic acid and L-5-methyltetrahydrofolate: Comparison of
495 clinical pharmacokinetics and pharmacodynamics. *Clin. Pharmacokinet.* 49, 535–548.

- 496 Samaniego-Vaesken, M.L., Alonso-Aperte, E., Varela-Moreiras, G., 2017. Voluntary folic acid
497 fortification levels and nutrient composition of food products from the Spanish market: A
498 2011–2015 update. *Nutrients* 9. <https://doi.org/10.3390/nu9030234>
- 499 Scaglione, F., Panzavolta, G., 2014. Folate, folic acid and 5-methyltetrahydrofolate are not the same
500 thing. *Xenobiotica* 44, 480–488. <https://doi.org/10.3109/00498254.2013.845705>
- 501 Stolzenberg-Solomon, R.Z., Chang, S.-C., Leitzmann, M.F., Johnson, K.A., Johnson, C., Buys,
502 S.S., Hoover, R.N., Ziegler, R.G., 2006. Folate intake, alcohol use, and postmenopausal breast
503 cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. *Am. J. Clin.*
504 *Nutr.* 83, 895–904. <https://doi.org/10.1093/ajcn/83.4.895>
- 505 Tapia-Hernández, J.A., Rodríguez-Félix, F., Katouzian, I., 2017. 9 - Nanocapsule formation by
506 electrospraying, *Nanoencapsulation Technologies for the Food and Nutraceutical Industries*.
507 Elsevier Inc. <https://doi.org/10.1016/B978-0-12-809436-5/00009-4>
- 508

List of captions:

Figure 1 The design of the storage experiment in buns. It included retention study during baking (1) and stability study during storage for 0, 3, 6, and 9 days (2). Three replicates were sampled at each sampling point. *indicate dough samples sampled for the calculation of the retention (n=3).

Figure 2 Scheme of the optimized electrospraying setup and conditions used in this study

Figure 3 SEM images and particle size distribution of electrosprayed capsules containing 1% Tween-20 surfactant after 3 Microfluidizer passes: (a) Capsules – Asc, (b) Capsules + Asc produced at optimum processing conditions (flow rate 0.006 mL/min, voltage 20 kV)

Figure 4 Recovery (%) of L-5-CH₃-H₄folate, free and in electrosprayed capsules during 21 days storage in darkness at room temperature (22°C). Different letters *abc* indicate significant differences between different samples within a day, whereas *uxyz* indicate significant differences within the samples analyzed on different days ($p \leq 0.05$).

Figure 5 Retention (%) of L-5-CH₃-H₄folate from buns enriched with L-5-CH₃-H₄folate, free or in capsules, during 9 days storage at room temperature (22°C) in darkness. Non-enriched buns represent 5-CH₃-H₄folate naturally occurring from the other ingredients in buns, whereas the other samples have added L-5-CH₃-H₄folate. Different letters *abc* indicate significant differences between different samples within the day, whereas *xy* indicate significant difference within the same sample between different days ($p \leq 0.05$).

Table 1 Chronological overview of the experiments used in optimization of electrospraying process and the results for the productivity

Table 2 Distribution of free and encapsulated L-5-CH₃-H₄folate in flour prior to dough preparation

Table 1 Chronological overview of the experiments used in optimization of electrospraying process and the results for the productivity

Exp.	Solutions	Asc (1%)*	Tween-20 (1%)**	L-5-CH ₃ - H ₄ folate (1%)*	Voltage [kV]	Flow rate [mL/min]	Productivity [mg/h]
1	15% glucose syrup + 4% pullulan	+	-	-	17	0.007	44.5
2	15% glucose syrup + 4% pullulan	+	-	-	16	0.007	0.9
3	15% glucose syrup + 4% pullulan	+	+	-	17	0.007	74.9
4	15% glucose syrup + 4% pullulan	+	-	-	16.5	0.007	29.7
5	15% glucose syrup + 4% pullulan	+	+	-	17	0.007	85.3
6	15% glucose syrup + 4% pullulan	+	+	-	20	0.006	63.1
7	15% glucose syrup + 4% pullulan	-	+	+	20	0.006	45.4
8	15% glucose syrup + 4% pullulan	+	+	+	20	0.006	73.9
9 [#]	15% glucose syrup + 4% pullulan	-	+	+	20	0.006	51.4
10 [#]	15% glucose syrup + 4% pullulan	+	+	+	20	0.006	55.9

Exp., experiment; Asc, ascorbic acid; L-5-CH₃-H₄folate, L-5-methyltetrahydrofolate.

* percentage of the compound in capsule

** percentage of the compound in biopolymer solution

[#]5 Microfluidizer passes

Table 2 Distribution of free and encapsulated L-5-CH₃-H₄folate in flour prior to dough preparation

Fortificant	Targeted L-5-CH ₃ -H ₄ folate concentration (µg/100 g) ^a	Required weight for enrichment (mg/kg)	Measured mean 5-CH ₃ -H ₄ folate (µg/100 g) ^b	Standard deviation (µg/100 g) ^b
Free L-5-CH ₃ -H ₄ folate	150	1.50	163 ^x	3.5
Capsules - Asc	150	150	155 ^x	19.2
Capsules + Asc	150	150	127 ^y	9.0

^a150 µg of L-5-CH₃-H₄folate/100 g of flour represent 1.5 mg/kg of flour fortification recommendation by FDA

^bSix determinations of each dry mix

Letters *xy* indicate significant difference tested by Tukey-Kramer ($p \leq 0.05$).

a)

b)

Highlights:

- Glucose-pullulan capsules containing L-5-CH₃-H₄folate were made by electrospraying
- Capsules are stable against oxidation during 21 days of storage in darkness at 22 °C
- Ascorbic acid added to capsules does not improve the stability of L-5-CH₃-H₄folate
- Encapsulation does not improve the retention of L-5-CH₃-H₄folate in buns

Author Contribution Statement:

The authors responsibilities were as follows: Petra Ložnjak Švarc, Pedro J. García-Moreno, Ana C. Mendes, Elnaz Z. Fallahasghari and Jette Jakobsen contributed to the design of the study; Elnaz Z. Fallahasghari performed electrospraying optimization, and examined physical properties of the capsules; Petra Ložnjak Švarc conducted stability studies including folate analysis, data analysis and wrote the manuscript. All authors helped in interpretation of data. Petra Ložnjak Švarc, Pedro J. García-Moreno, Ana C. Mendes, and Jette Jakobsen read, reviewed and approved the final manuscript.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

--