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Impact of three warming-up methods on the stability of vitamin C and 5-methyltetrahydrofolate supplemented to apple and carrot purée

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A B S T R A C T

Two methods that are used at people's home to warm-up food namely a microwave and an Actifyr® device, and a system that is usually employed in canteen kitchens to keep food warm, were examined on their impact on the stability of vitamin C and 5-methyltetrahydrofolate. The purée was heated until ebullition with the microwave, which lasted 1.5 min. Purée which was warmed-up by the Actifyr® device needed around 20 min to attain 80 °C. When the purée was kept warm by a water bath, the vitamin stability was pursued for 180 min at 80 °C. During the latter, stability was examined at the surface and bottom of recipients that were filled with 600 g or 200 g of purée corresponding to a filling height of 5 cm and 1.5 cm respectively. Although vitamin C and 5-methyltetrahydrofolate are usually referred to as heat- and oxygen-susceptible molecules, none of the methods led to major vitamin losses. In terms of the warm holding method, no difference was observed between withdrawals at the surface and the bottom of vessels for neither of the two filling levels. Vitamins were also fairly stable at the surface of recipients where oxygen exposition is high.

Keywords:

Reheating
Warm holding
Ascorbic acid
Folate
Supplementation

1. Introduction

Vitamin C and vitamin B₉, the latter is also known as folate(s), are essential micronutrients which have to be ingested as they cannot be synthesized by humans' metabolism. Vitamin supplementation can be used to increase their intake. Folic acid, a synthetic folate vitamer, is predominantly used for enrichment of folates however it might mask vitamin B₁₂ deficiency. Thus, the natural abundant vitamer, 5-methyltetrahydrofolate, has been proposed as alternative for supplementations (Pietrzik, Bailey, & Shane, 2010; Scaglione & Panzavolta, 2014). It is susceptible to deterioration though. To comply with the intention of enrichments, that is to supply a certain vitamin amount, the stability study of vitamin C and 5-methyltetrahydrofolate is indispensable.

Vitamin C and 5-methyltetrahydrofolate are both sensitive to oxygen and temperature (Delchier et al., 2014; Dhuique-Mayer et al., 2007). When oxygen is absent, no degradation of vitamin C and 5-methyltetrahydrofolate occurs at intermediate temperatures i.e. between 40 °C and 80 °C (Delchier et al., 2014; Herbig,

Maingonnat, & Renard, 2016). Up to 100 °C, oxygen is still soluble and in dynamic equilibrium with headspace oxygen (Van Bree et al., 2012). Dissolved oxygen in supplemented apple and carrot purée is consumed entirely within 1 h at 80 °C in contrast to model solutions (Herbig et al., 2016). After the depletion, oxygen is only available in the headspace. The filling height and thus oxygen availability is the factor that influences predominantly the stability of vitamin C in apple purée serum that is heated at 80 °C in Eppendorf tubes what can be ascribed to different surface-to-volume ratios (Herbig & Renard, 2017).

Unfortunately, 5-methyltetrahydrofolate is very unstable in contrast to folic acid, and degrades rapidly in model solution (Delchier et al., 2014; Paine-Wilson & Chen, 1979). By adding ascorbic acid, complete stability of 5-methyltetrahydrofolate can be attained during heat treatments (Herbig, 2016; Oey, Verlinde, Hendrickx, & Van Loey, 2006). However, the amount of ascorbic acid which is necessary for total protection cannot be generalized, for two reasons. First, even if ascorbic acid is available in molar excess compared to the amount of dissolved oxygen or 5-methyltetrahydrofolate, degradation starts after an initial protection period (Herbig, 2016). And second, the amount can be supposed to depend on the overall oxygen availability which is influenced by the experimental set-up. The exposition to headspace oxygen, which is determined by the geometry of the recipient

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and increases when the medium is stirred, may play a crucial role. Nevertheless, the time length of complete protection of 5-methyltetrahydrofolate can be extended by increasing the vitamin C amount (Herbig, 2016).

Reheating of food exhibits other constraints than cooking in terms of temperature and time length. Temperature recommendations for hot keeping of food are very similar in different European countries with a minimum temperature of 63 °C in France (Direction de l'information légale et administrative, 2013) and 65 °C in Germany (Bundesinstitut für Risikobewertung, 2008). The aim of this recommendation is to impede growth of spore-forming bacteria. In terms of heating length, it has to be distinguished between people's homes and canteen kitchens. At the latter, food is usually held warm up to several hours.

The impact of warming-up methods on the stability of vitamin C and 5-methyltetrahydrofolate has rarely been studied. Data exist for microwave heat treatments. Depending on the heating length and the type of food, vitamins are preserved or lost. For example, only 2% of vitamin C in strawberry purée were lost when the purée was treated at 90 °C for 10 s and 12% when it was heated at 120 °C for 10 s under the conditions of Marszałek, Mitek, and Skapska (2015). It was not lost in kiwi purée after a treatment at 1000 W for 340 s (Benlloch-Tinoco, Igual, Salvador, Rodrigo, & Martínez-Navarrete, 2014) and to 46% in broccoli which stayed at 1000 W for 5 min (Vallejo, Tomas-Barberan, & Garcia-Viguera, 2002). Bureau et al. (2015) observed that vitamin C and folates in 13 frozen vegetables without addition of water were well retained after microwaving them between 10 and 18 min. Johansson, Furuheggen, Frolich, and Jagerstad (2008) compared the impact of microwave heating (900 W/5.5 min), reheating in a saucepan until food reached 85 °C and warming-up in an oven (225 °C/40 min) on folates stability in 10 precooked vegetarian food products. All methods significantly influenced the stability of folates but variability of each method was high and thus no recommendation could be inferred.

At people's homes, microwaves are often employed. Warming-up with an Actifyr® device, which is usually used to cook French fries with a reduced amount of oil, could be an alternative. The Actifyr® device is equipped with a scoop which automatically stirs the food product. Heating is carried out via conduction and additionally by ventilation of hot air in the headspace. Each heating method has its own, critical parameter for vitamin losses. When food is warmed-up by a microwave, heating until ebullition is necessary to ensure food safety. When using an Actifyr® device, the product is heated at a lower temperature but is stirred and thus more exposed to headspace oxygen. In canteen kitchens, duration can be a critical factor as food is usually kept warm up to several hours.

The objective of the present work consisted in assessing the stability of vitamin C and 5-methyltetrahydrofolate after a warming-up treatment carried out by three different systems. It was envisaged to optimize the heating protocols in case that vitamins are lost. Apple and carrot purée was therefore supplemented with the two vitamins on a pilot scale. The impact of a microwave and an Actifyr® device, and that of a warm holding method which is usually used in canteen kitchens, was studied. When examining the impact of the latter method, special attention was paid on the effect of filling height of recipients and degradation at the bottom and surface of vessels.

2. Material and methods

2.1. Production and supplementation of apple and carrot purée

Supplemented apple and carrot purée (25 kg respectively) were

produced by the "Centre Technique de la Conservation des Produits Agricoles" (CTCPA) in Avignon/France. In Fig. 1, production steps are shown. For the preparation of apple purée, apples of the type "Golden" were washed and cut into pieces. Afterwards, apple pieces were chopped at 80 °C for 1 h in a "Stephan" (Stephan, Hameln, Germany) which is a combination of a mixer and a cooker. The apple mix was passed through a "Parmentière" (Eillert, Ulf, Netherlands) that is a filter, to discard apple cores and seeds. An amount of 25 kg of the purée was weighed, let cool down for 30 min and subsequently homogenized for 10 min in the "Stephan". For the production of enriched carrot purée, the same procedure as for apple purée was applied except that the washing and cutting step, and filtration by the "Parmentière" were omitted since deep-frozen carrot pieces were used.

Afterwards, ascorbic acid and 5-methyltetrahydrofolate were supplemented. Therefore, ascorbic acid (12.625 g) and 5-methyltetrahydrofolate (50.5 mg) were dissolved in 250 mL ultra-pure water. The aimed ascorbic acid concentration in the purée was 50 mg/100 g and of 5-methyltetrahydrofolate 0.2 mg/100 g. The solution with the two vitamins was poured in the purée. Afterwards, the mixture was stirred for homogenization during 10 min in the "Stephan". The supplemented purée was transferred into cans which were deep-frozen (≤ -18 °C) until experimentation.

2.2. Reheating experiments

Purées were thawed overnight in a cold room (8 °C) before each heat treatment. Temperature in the reheating vessels and after microwave was monitored using a digital thermometer (EU 620-0916NA82020-744, VWR, Fontenay-sous-bois, France).

2.2.1. Microwave warming-up

Thawed purée (250 g, corresponding to one can) was poured in a deep plate. A domestic microwave of the type Crisp FT439 Whirlpool (Whirlpool Corporation, Michigan, USA) was used for the heat

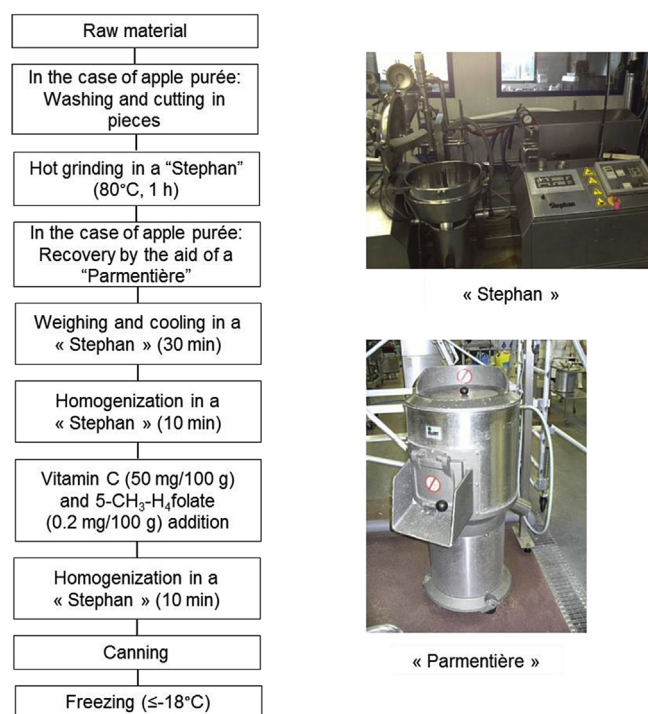


Fig. 1. Processing steps of supplemented apple and carrot purée.

treatment. The purée was heated until ebullition which took 1 min and 30 s at 1300 W. One aliquot per plate of approximately 1 g was taken before and after the heat treatment and deep-frozen (≤ -18 °C).

2.2.2. Actifry® heating

Purée (1 kg) was weighed in the device. The integrated scoop of the Actifry® device (Groupe SEB, Ecully, France) turned with a pace of 2 rounds/min. The temperature of the medium was followed continuously and the heat treatment was stopped when 80 °C were reached, which was achieved in about 20 min. Aliquots before and after the heat treatment of approximately 1 g were withdrawn for vitamin analyses and immediately deep-frozen (≤ -18 °C).

For determination of moisture contents, samples stayed for 4 d at 70 °C in drying cabinets.

2.2.3. Warm holding method

A water bath of the type Royal Catering, RCBM 1/6150 GN (Royal Catering Corporation, Chemnitz, Germany) was employed, which was endowed with six standardized 1/6150 GN recipients (width x length x depth: 176 mm x 162 mm x 150 mm) and six cover lids with gaskets that hindered water evaporation. Each vessel corresponded to one time point. The purée was preheated up to 80 °C in a pan before it was transferred to the six recipients. Temperature of the device was fixed at 80 °C. The first point of a time curve corresponded to an aliquot which was withdrawn after the preheating step. During the heat treatment of 3 h, samples were regularly withdrawn at the surface and the bottom. The effect of the filling height was investigated by using 600 g or 200 g of purée for one vessel, which corresponded to a filling height of approximately 5 cm and 1.5 cm.

2.3. Vitamin C measurement

The method of Stevens, Buret, Garchery, Carretero, and Causse (2006) was employed which is based on spectrophotometric detection of both reduced (ascorbic acid) and oxidized form (dehydroascorbic acid) of vitamin C. Aliquots of 500 mg were taken for analysis. A spectrophotometer (Safas Xenius, Monaco) was used for absorption measurements at 525 nm. Vitamin C was calculated as the sum of ascorbic and dehydroascorbic acid.

2.4. 5-Methyltetrahydrofolate analysis

The method of Ndaw, Bergaentzle, Aoude-Werner, Lahely, and Hasselmann (2001) was applied. A HPLC connected to a fluorescence detector (RF-10AXL, Shimadzu Inc., Kyoto, Japan) was employed for analysis. The excitation wavelength was set at 295 nm and the emission wavelength at 356 nm. A guard column (LiChrospher RP18 All Guard, 7.5 x 4.6 mm, Alltech, Epernon, France) and a LiChrospher 100 RP18 column (250 x 4.5 mm, 5 µm, Alltech, Epernon, France) were used. The mobile phase consisted in acidified water (1 mL/L, formic acid) and acetonitrile. The gradient at the beginning was 5% acetonitrile which was increased linearly to 58% within 14 min and then to 100% in 3 min. It was held for 11 min at 100%, then decreased to 5% acetonitrile in 2 min and was subsequently kept at 5% for 5 min. The flow rate was 0.8 mL/min and the injection volume 25 µL. An external calibration was carried out. 5-Methyltetrahydrofolate was purchased at Schircks (Jona, Switzerland).

3. Results

3.1. Test of homogeneity

Homogenous distribution of supplemented vitamin C and 5-methyltetrahydrofolate within the apple and carrot purée batch was verified. Two aliquots, one at the surface and another at the bottom, of five respective cans were therefore withdrawn.

The vitamin C contents in apple and carrot purée, at the two locations of all cans gave almost identical values (Fig. 2). The average concentration of all ten analyzed vitamin C samples, accounted to 51 ± 2 mg/100 g in apple purée and to 47 ± 2 mg/100 g in carrot purée. The measured concentration corresponded thus to the amount that was added. In addition, homogeneity in both matrices was indicated by low standard deviations.

5-Methyltetrahydrofolate was, as vitamin C, equally distributed in apple purée (Fig. 2). The mean value of all analyzed contents was 141 ± 9 µg/100 g. The determined amount was lower than the

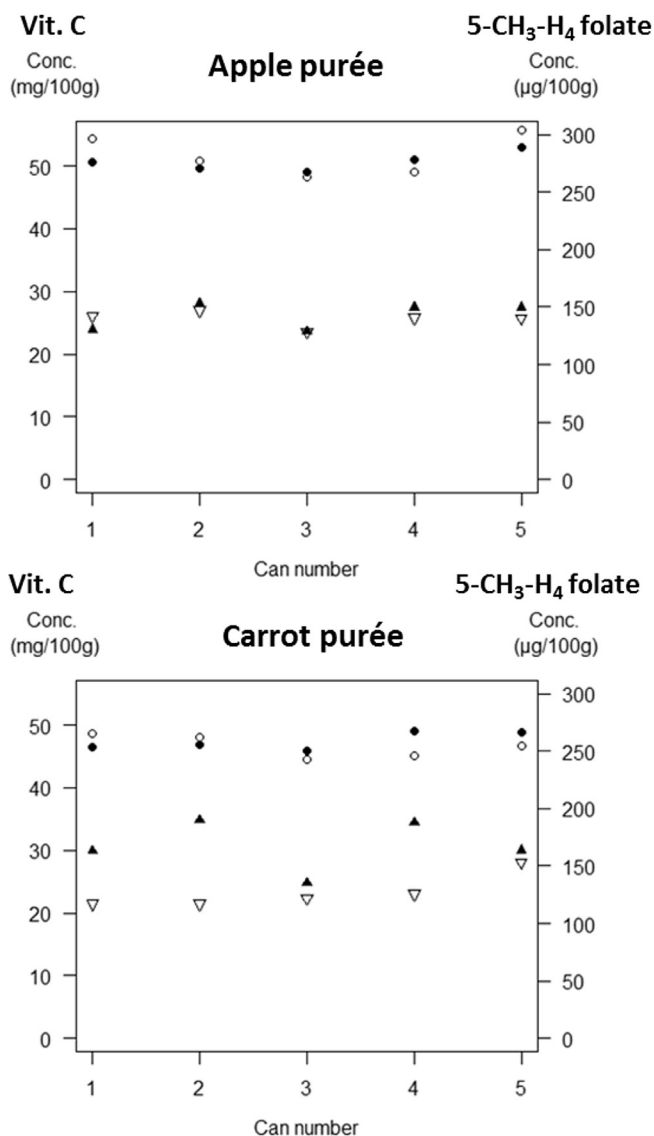


Fig. 2. Test of vitamin homogeneity in apple and carrot purée. Aliquots were taken at the surface and at the bottom of five respective cans. Vitamin C content at the surface (○) and at the bottom (●). 5-CH₃-H₄folate content at the surface (▽) and at the bottom (▲).

added content of 200 µg/100 g. Nevertheless, the low standard deviation showed that 5-methyltetrahydrofolate was homogeneously distributed within apple purée. In carrot purée, contents at the surface of cans were slightly lower than at the bottom (Fig. 2). The mean concentration of all contents withdrawn at the surface of cans was 127 ± 15 µg/100 g and that at the bottom was 168 ± 22 µg/100 g. The average concentration of all aliquots was 147 ± 28 µg/100 g. The difference between the added amounts and the final concentrations could be due to losses during the mixing step. An accumulation of non-soluble particles (from plant cell walls origin) in the surface region might have led to the lower 5-methyltetrahydrofolate concentration in carrot purée. The concentration discrepancy between the two locations was however not enormous, and the differences between cans limited. Therefore before starting heat treatment experiments, purées were homogenized again by the aid of a spoon to reduce this inhomogeneity.

3.2. Microwave warming-up

Supplemented apple and carrot purée were heated until ebullition which lasted 1.5 min at a power of 1300 W. After the heat treatment, the purée was homogenized by a spoon and samples were withdrawn. Vitamin C and 5-methyltetrahydrofolate withstood the treatment without major losses (Table 1). High stability of vitamin C when food is heated by a microwave is in accordance with the result of Benlloch-Tinoco et al. (2014), Marszalek et al. (2015) and Bureau et al. (2015).

3.3. Actifry® heating

The Actifry® device heated apple purée slightly quicker than carrot purée. The aimed temperature of 80 °C was attained in apple purée 17 and 18 min whereas warming-up of carrot purée lasted 19 and 21 min. Water was significantly lost during the treatment as evidenced by a weight loss of 18 ± 1% in apple purée and of 23 ± 3% in carrot purée. This loss was determined by weighing the device before and after the treatment. However, evaporation appeared to occur only at the surface which was indicated by formation of a crust-like, dried layer of purée only at the top and water contents inside the product which did not change. In apple purée, the moisture content before the treatment accounted to 72.3 ± 6.6% and afterwards to 72.1 ± 6.1%, and in carrot purée to 86.9 ± 1.7% before, and to 81.1 ± 6.2% after the treatment. This result indicates that homogenization of purée during the heat treatment was weak. Under these conditions minor to negligible degradation of both vitamins was determined (Table 2).

3.4. Warm keeping method

After preheating the purée in a pan and transferring it to the vessel, the temperature first decreased (Fig. 3). The regulated temperature of 80 °C was attained again after around 1 h, but only at the bottom of vessels not at the surface. At the same position, no difference between the temperature time curve in apple and carrot purée was observed (Fig. 3). When recipients were filled with 600 g

Table 1
 Concentration of vitamin C and 5-CH₃-H₄folate before and after the microwave heat treatment. Means of duplicates are shown.

| Matrix | Time (min) | Vit. C (mg/100 g) | 5-CH ₃ -H ₄ folate (µg/100 g) |
|--------------|------------|-------------------|---|
| Apple purée | 0 | 52.1 ± 1.1 | 229 ± 6 |
| | 1.5 | 52.6 ± 0.4 | 226 ± 6 |
| Carrot purée | 0 | 46.0 ± 4.2 | 210.2 ± 0.4 |
| | 1.5 | 41.5 ± 0.3 | 212 ± 4 |

Table 2
 Concentration of vitamin C and 5-CH₃-H₄folate before and after the Actifry® heat treatment. Means of duplicates are shown.

| Matrix | Time | Vit. C (mg/100 g) | 5-CH ₃ -H ₄ folate (µg/100 g) |
|--------------|-----------|-------------------|---|
| Apple purée | initially | 44.0 ± 2.7 | 246 ± 30 |
| | end | 43.6 ± 1.0 | 184 ± 63 |
| Carrot purée | initially | 32.1 ± 2.9 | 203 ± 41 |
| | end | 33.2 ± 0.2 | 232 ± 10 |

of purée, the mean temperature at the bottom between 60 and 180 min was 81 ± 1 °C in apple purée and 81 ± 2 °C in carrot purée. At the surface, the average temperatures were of 64 ± 3 °C and of 64 ± 3 °C respectively. When vessels were filled with 200 g, the same tendency was observed. The mean temperature at the bottom was 78 ± 1 °C in apple purée and 78 ± 4 °C in carrot purée, and at the surface 64 ± 5 °C and 68 ± 7 °C respectively.

Even if a temperature difference between bottom and surface

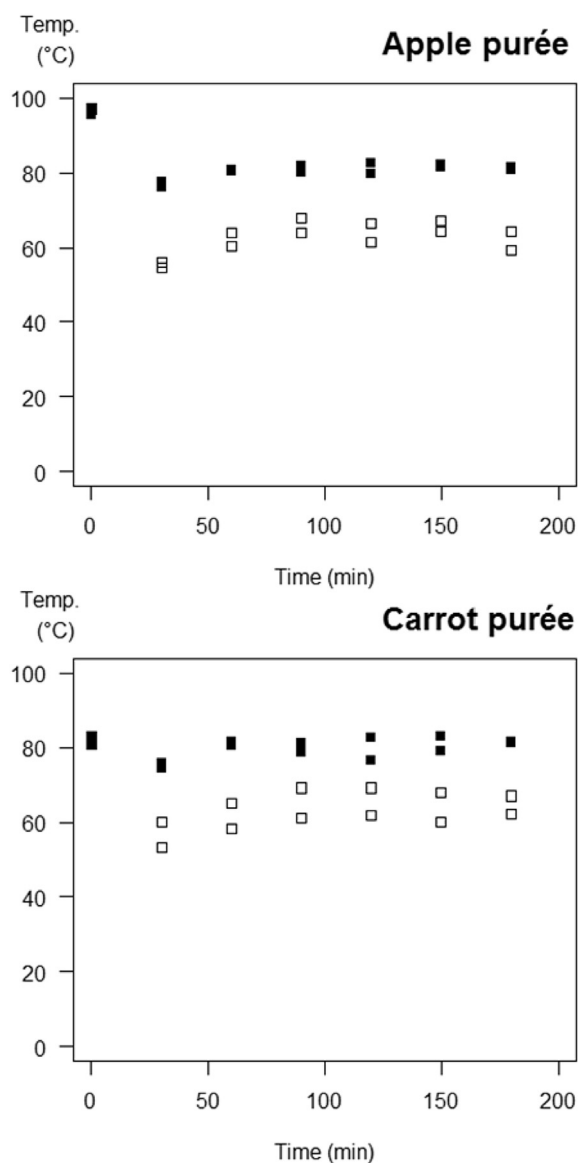


Fig. 3. Temperature time courses in apple and carrot purée during warm holding. Measured at the bottom (■) and at the surface (□) of vessels that were filled with 600 g of purée.

positions of around 15 °C existed, the respective temperature was, after the initial decrease, stable over time and also at the surface still in accordance to food safety regulations. Degradation rates of vitamin C increase non-significantly with temperature between 60 and 80 °C (Herbig & Renard, 2017). It could thus be excluded that the temperature difference in the two locations influenced degradation paces. It was however supposed that as a consequence of oxygen consumption in the medium and faster oxygen renewal near the surface by oxygen coming from the headspace, the degradation rate at the surface is higher than that at the bottom. To verify this, vitamin C and 5-methyltetrahydrofolate contents were analyzed at the surface and the bottom of recipients filled with 600 g or 200 g of purée.

In apple purée, slight degradation of vitamin C was observed in the course of time. However, the degradation pace at the surface did not differ from that at the bottom of vessels (Fig. 4) and the filling volume did not impact the degradation either. The rate of degradation was identical when recipients were filled with 200 g or 600 g (Fig. 4).

In carrot purée, initial values were already at the beginning much lower than the supplemented amount of 50 mg/100 g (Fig. 4). This might be due to vitamin C degradation during storage since when homogeneity was tested, values still corresponded to the enriched amount. Although the initial content was lower than the supplemented amount, the heat treatment did not lead to vitamin C

degradation (Fig. 4). As for apple purée, no impact of the position where the aliquot was taken, or the filling height was established.

Vitamin C degrades also on a lab-scale level slightly faster in apple than in carrot purée (Herbig & Renard, 2017). No difference between degradation rates was determined when vessels were filled with 200 g or 600 g which corresponded to a filling height ratio of around 1:3. The impact of filling height which was ascertained on the lab-scale level (Herbig & Renard, 2017) could not be confirmed on the scale-up level. In addition, stability was homogenous within the medium. Vitamin C did not degrade faster near the surface, contrary to what has been observed for agar gel during storage at 20 °C (Penicaud, Broyart, Peyron, Gontard, & Guillard, 2011). An impact of the sample location in vessels on the degradation rate might however also appear at elevated temperatures when degradation is in an advanced state.

Results concerning the stability of 5-methyltetrahydrofolate were less evident as a consequence of high standard deviations, especially in carrot purée (Fig. 4). Already initial values varied quite strongly. Less disparity of values had been observed when homogeneity within purées was tested or when the effect of a microwave or an Actify® heat treatment was examined. The variability of contents could not be ascribed to withdrawal at the surface or bottom position either, as values were randomly distributed (Fig. 4). In addition, low variation of values was observed when the concentration of vitamin C in the course of time was analyzed. The

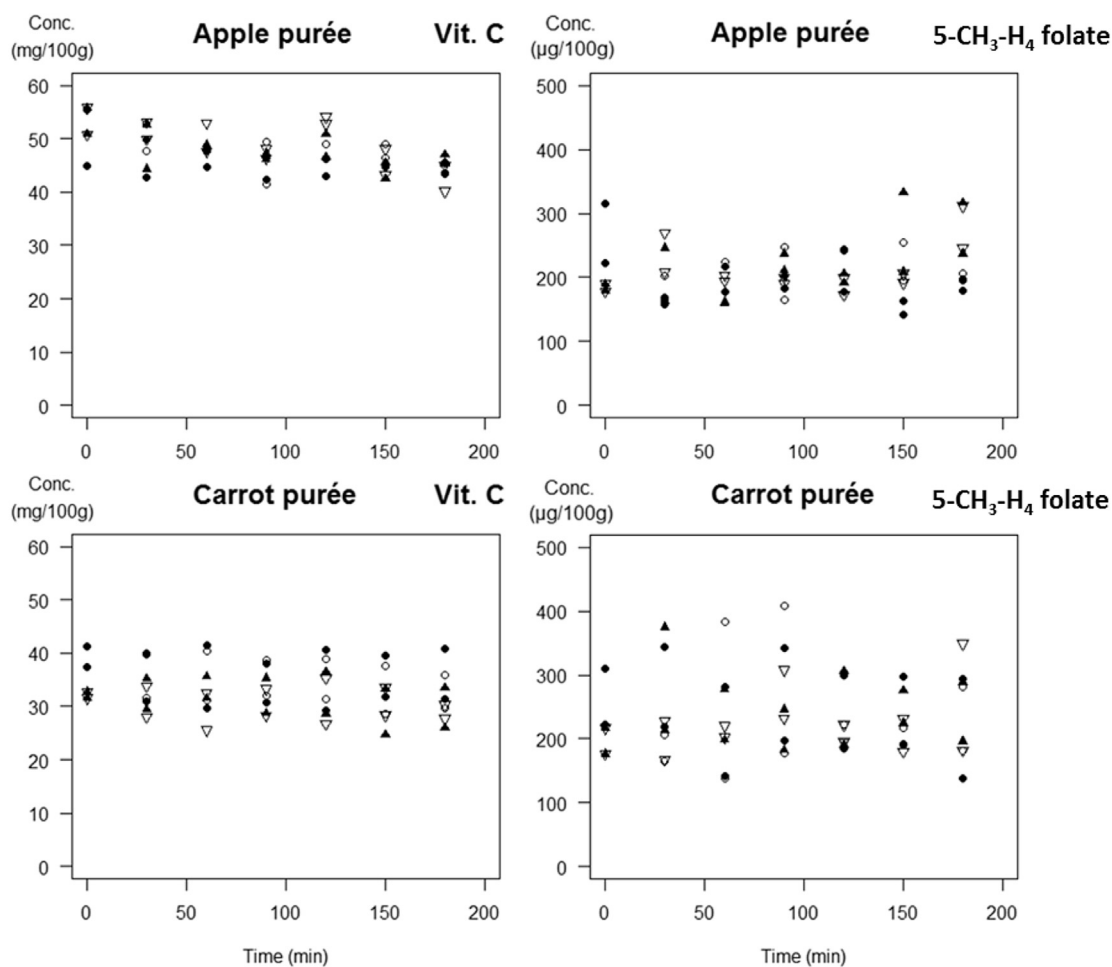


Fig. 4. Concentration of vitamin C and 5-methyltetrahydrofolate (5-CH₃-H₄ folate) in apple purée and carrot purée during warm holding. Measured at the surface (○) and at the bottom (●) of vessels that were filled with 600 g of purée, and at the surface (▽) and at the bottom (▲) of vessels that were filled with 200 g of purée.

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high variability might thus result from the preheating phase which impacted only the concentration of 5-methyltetrahydrofolate.

In apple purée, the average of all initial contents was 227 ± 62 $\mu\text{g}/100$ g and the mean of final amounts 236 ± 53 $\mu\text{g}/100$ g. In carrot purée, the mean of contents accounted to 231 ± 57 $\mu\text{g}/100$ g and to 239 ± 74 $\mu\text{g}/100$ g respectively. It appeared that 5-methyltetrahydrofolate was still, fairly stable.

4. Conclusion

Vitamin C and 5-methyltetrahydrofolate are commonly described as fragile molecules that are very sensitive to oxygen and temperature. The results of this study show however, that when they are supplemented together to apple or carrot purée, both vitamins are quite stable during warming-up of products. They were not or to a negligible amount degraded when heated by a microwave, an Actifry® device, or when held warm for 3 h. During the latter, stability was pursued at surface and bottom of the medium but no stability difference was ascertained. Even if exposition to headspace oxygen was high at the surface, this did not impact the vitamin stability. Modeling of vitamin losses or adaption of initial vitamin amounts, are thus not necessary. The vitamin amount that is enriched, is also maintained even if the product is reheated.

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