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Susceptibility of 5-methyltetrahydrofolic acid to heat and microencapsulation to enhance its stability during extrusion processing

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ABSTRACT

Folic acid is a common form of folate used for food fortification to prevent the incidence of neural birth defects among others. However, 5-methyltetrahydrofolic acid (5-MTHF) is considered, a better alternative to folic acid and is less likely to mask the symptoms of B_{12} deficiency in older populations. Also it is less bioavailable, and present in very low amount in foods. Fortification of foods with 5-MTHF is considered problematic, because it is highly sensitive to normal food processing operations. A comparative study on the thermal stability of folic acid and 5-MTHF in various liquid model food matrices such as milk, soymilk, starch–water and water during boiling and autoclaving at various time intervals was performed. Thermal degradation of 5-MTHF was more severe than folic acid in all food matrices e.g., almost 70% loss of 5-MTHF *vs* 17% loss of folic acid. Microencapsulation of 5-MTHF is considered to improve the stability of 5-MTHF during processing. A combination of pectin (P) and sodium alginate (A) at three different proportions (P60:A40, P70:A30, P80:A20) were employed for encapsulating 5-MTHF by spray drying. Microcapsules with the highest loading efficiency of 60% of vitamin (from P80:A20) and unencapsulated 5-MTHF powder was incorporated into starch and extruded at six different temperatures (100, 110, 120, 130, 140, 150 °C). The 5-MTHF had better stability (84–94.5% retention) compared to the free form (65.3–83.2%) in all extruded products. The effectiveness of encapsulation was more evident at higher extrusion temperatures.

1. Introduction

Folate acts as a coenzyme substrate in many reactions of amino acids and nucleotides. The reduced forms of folates serve as an acceptor or donor of a single carbon unit, a reaction collectively called 'single carbon metabolism'. The presence of genetic defects in normal folate-dependent metabolic processes involving DNA synthesis, has been linked to cancer initiation (Fenech, 2001). Folate deficiency in the diet has been linked to malformation of the embryonic brain/spinal cord development, a condition referred to as *neural tube defects or NTDs* (manifested by still-birth, mental retardation, swollen head, and poor bladder control) (Czeizel & Dudas, 1992; Medical Research Council (MRC) Vitamin Study Research Group, 1991). Recent data has showed that there are around 338 incident cases of NTDs a year in Australia (FSANZ, 2006). Folate deficiency is also linked to the accumulation of plasma homocysteine levels, a strong predictor of carotid artery narrowing which is a predictive risk for both stroke and atherosclerosis (Selhub et al., 1995). Folate exists in a significant amount in green leafy vegetables, fruits, legumes, fermented vegetables, egg yolk while most cereals and their flours are poor sources of folate (Shrestha, Arcot, & Paterson, 2003). Considering the significant number of NTD cases, fortification of cereal based foods with folic acid (synthetic form) has been made mandatory in 57 countries including the USA and Australia.

Among 100 folate compounds, folic acid and 5-methyl-5,6,7,8-tetrahydrofolic acid, are the most commonly reported forms (Fig. 1, Gregory, 1989). The presence of folic acid is almost non-existent in plant and animal foods whereas 5-MTHF, 5-formyltetrahydrofolate, 10-formyltetrahydrofolate and other reduced folates are more abundant (Ginting & Arcot, 2004). Bioavailability of folic acid from fortified foods is reported to be different from naturally available folates (Wright, King, Wolfe, Powers, & Finglas, 2010). The use of folic acid as a fortificant is often considered controversial as it can mask the haematological abnormalities of vitamin B_{12} deficiency while the neurological complications remain in progress (NHMRC, 1995). There is now consideration for the use of 5-MTHF which cannot possibly mask the Vitamin B_{12} deficiency as an alternative to folic acid (Wright, Finglas, & Southon, 2001). Folic

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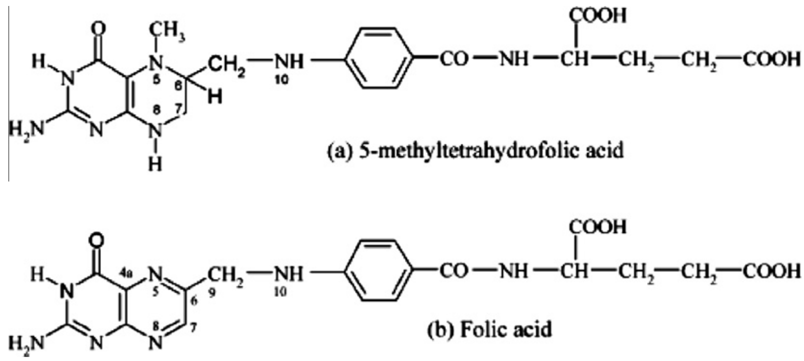

Fig. 1. Chemical structures of 5-methyltetrahydrofolic acid and folic acid (Arcot & Shrestha, 2005).

acid and the pure natural isomer 6S-5-MTHF have been found to have similar bioavailability (Pentieva et al., 2004). Historically, 5-MTHF has not been considered as a folate fortificant as it has been difficult and expensive to synthesize and less stable. All folate compounds are sensitive and easily destroyed under high temperatures, air, light, low pH, and reducing agents. Various studies have shown that 5-MTHF is more sensitive to thermal degradation when compared to folic acid (Indrawati, Ottoy, Loey, & Hendrickx, 2004b; Indrawati et al., 2004a; Nguyen, Indrawati, & Hendrickx, 2003) and easily loses vitamin activity during food processing. There have been a number of studies where thermal degradation kinetics of folic acid and 5-MTHF was determined under various conditions e.g., presence at various levels or absence of oxygen, (Virberg, Jagerstad, Oste, & Sjorhold, 1997); high hydrostatic pressure (Indrawati et al., 2004a; Nguyen et al., 2003); and pH, buffer type and presence of antioxidants (Indrawati et al., 2004b; Mnkeni & Beveridge, 1983).

Several studies have reported significant losses of folic acid in fortified foods during processing (Arcot, Shrestha, & Gusanov, 2001; Osseyi, Wehling, & Albrecht, 1998; Shrestha et al., 2003). So far, very few studies have been done to evaluate the effect of processing on 5-MTHF fortified foods. Cereal based extruded foods and liquid drinks such as flavoured starch based drinks, dairy and soy milks, fruit juices, carbonated drinks etc., are commonly used as vehicles for folic acid fortification. Liquid foods are plausible alternatives to improving the bioavailability of folate compounds instead of grains. Liquid foods have easy passage to the digestive system since they do not depend on rigorous mechanical digestion in the gut and are easily absorbed through blood. Besides, liquids as delivery vehicles are a better option since they can be homogenously distributed in solution compared to solid foods. Extrusion is a thermo-mechanical treatment which is extensively used as a processing method for many fortified cereal based foods. One possible adverse effect of feed and food processing through extrusion is destruction of vitamins. Riaz, Asif, and Ali (2009) have reviewed the effect of extrusion on stability of vitamins where folic acid is reported to undergo significant degradation.

It is expected that processing foods at elevated temperatures, pressure and exposure to air and light may cause significant loss of 5-MTHF. The knowledge of retention/loss of folate in fortified processed foods is important to ensure adequate 5-MTHF is present in target foods (for manufacturers as well as consumers). This information would also help to predict the final concentration of 5-MTHF in processed foods. It is also realized that there is an urgent need to develop newer technique(s) to improve the stability as well as bioavailability of added synthetic folate vitamers in foods. Encapsulation is one of such techniques where the target substance is confined within a polymeric matrix coated by one or more semi-permeable polymers, by virtue of which the encapsulated compound becomes more stable than isolated or free form (Dziezak, 1988; Madziva, Kailasapathy, & Phillips, 2006). Encapsulation has been used extensively to entrap flavouring compounds, drugs, polyunsaturated and bioactive compounds and control their release into the gastrointestinal tract (Polk et al., 1994). A number of studies have shown that microencapsulation of folic acid improves its stability during food processing (Biodar, 2000; Madziva, Kailasapathy, & Phillips, 2005; Madziva et al., 2006; Marchetti, Tossani, Marchetti, & Bauce, 1999). Our previous work on coating of folic acid fortified rice with edible polymers such as pectin, locust bean gums and others (in solution) showed a much higher retention of folic acid during washing and cooking (Shrestha et al., 2003).

Spray drying is one of the most common and cheapest methods to produce encapsulated flavour as well as achieve the addition of sensitive micronutrients through fortification. So far, there is no study on microencapsulation of 5-MTHF and its stability during extrusion processing. The major aims of current studies are: to evaluate the stability 5-MTHF in different simulated liquid food systems processed under different heating conditions; microencapsulation of 5-MTHF in pectin–alginate gel; and evaluation of encapsulated vitamin under extrusion conditions.

2. Materials and methods

2.1. Materials

Soymilk and low fat milk were purchased from the local supermarket. Normal maize starch (27% amylose) was purchased from Penfords Australia, Sydney, Australia. Folic acid (PteGlu-Na_2) and 5-MTHF [(6R,S)-5-methyl-5,6,7,8-tetrahydrofolic acid calcium salt] were obtained from Schricks Laboratories, Jona, Switzerland. Milli-Q Grade, distilled deionized water was used throughout the analysis.

Standard solutions were prepared under subdued conditions. Brown bottles were used to store vitamin solutions. In the case of transparent glass containers, aluminium foil was wrapped to prevent direct sunlight exposure. The purity of each folate standard was checked using a spectrophotometer at appropriate wavelengths (Blakley, 1969) with a pH 7 phosphate buffer as the blank. All other reagents were of analytical grade, purchased from local suppliers.

Stock solution of folic acid and 5-MTHF (1 mg/ml in 0.01 M NaOH in 20% ethanol) was flushed with nitrogen. The solution was distributed in a number of small brown bottles, about 10 ml capacity, and flushed with nitrogen and stored at −18 °C until further use. For the HPLC calibration curve, standard solutions of both folate vitamers (200 ng/ml in 0.1 M acetate buffer or elution buffer) were prepared on the day of HPLC analysis from stock solution.