Food Chemistry 210 (2016) 182–188

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Culinary practices mimicking a polysaccharide-rich recipe enhance the bioaccessibility of fat-soluble micronutrients

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ARTICLE INFO

Article history:
Received 20 October 2015
Received in revised form 11 April 2016
Accepted 13 April 2016
Available online 28 April 2016

Keywords:
Carotenes
α-tocopherol
Digestion model
Cooking process
Tunisian mloukhiya
Corchorus olitorius

ABSTRACT

This study was carried out to assess the impact of heat processing of a complex emulsion on the behavior of fat soluble micronutrients (FSM) in a traditional Tunisian dish. A simplified recipe involved, dried mucilage-rich jute leaves, tomato paste and olive oil, followed by a cooking treatment (150 min). Hydrothermal pattern and viscosity were monitored along with the changes of FSM content and the bioaccessibility (called micellarization, using an *in vitro* digestion model). Partitioning of carotenoids differed according to their lipophilicity: lycopene, β-carotene and lutein diffused to the oil phase (100%, 70% and 10% respectively). In contrast with the poor carotenes/tocopherol bioaccessibility (0.9–1%), the highest micellarization was observed for lutein (57%) and it increased with heating time and viscosity change. Domestic culinary cooking practices probably increase the bioavailability of carotenes mainly by their diffusion to the oil phase, facilitating their *in vivo* transfer into micelles.

1. Introduction

The healthy effects of the Mediterranean diet could be related to higher intake of fibers, olive oil, vitamins and bioactive compounds such as polyphenols and carotenoids (Romaguera et al., 2011; Sofi, Macchi, Abbate, Gensini, & Casini, 2013). Among bioactive compounds, fat soluble micronutrients (FSM) such as pro- and non provitaminic A carotenoids and tocopherols (vitamin E) have been reported to be dietary markers related to the healthy or protective effects of the Mediterranean-like diet (Olmedilla et al., 2001; Witkowska, Zujko, & Mironczuk-Chodakowska, 2013). With the aim of promoting traditional Mediterranean foods that could have a positive nutritional impact in public health, it is essential to study the behavior of these FSM during processing, formulation and the first steps of digestion.

Indeed, the FSM content is highly dependent of the processing and formulations conditions. The influence of thermal processing on the behavior of both carotenoids and tocopherols has only been investigated by a few authors for tomato-derived products and other vegetables (Bernhardt & Schlich, 2006; Hwang, Stacewicz-Sapuntzakis, & Bowen, 2012; Seybold, Fröhlich, Bitsch, Otto, & Böhm, 2004). Overall, α-tocopherol and lycopene were quite thermally stable whereas β-carotene was slightly affected by industrial processing of tomato-based products. Lycopene isomerization and loss occurred especially when incorporated in model systems such as oil emulsions or solvents (Chanforan, Loonis, Mora, Caris-Veyrat, & Dufour, 2012; Colle et al., 2010; Koh, Charoenprasert, & Mitchell, 2012).

Otherwise, few authors have quantified the effects of process parameters on carotenoid bioaccessibility. The bioaccessibility of a FSM such as carotenoids is the proportion released from food matrix and solubilised into micelles and therefore available for absorption by intestinal cells while the bioavailability (*in vivo*) is defined as the fraction of ingested micronutrients absorbed and delivered to the target tissues for utilization or storage (Reboul et al., 2006). It is assumed that heat treatments can modulate carotenoids bioaccessibility via disruption of plant cell walls leading to the release of these fat-soluble compounds from protein complexes (Castenmiller & West, 1998). Lycopene and β-carotene bioaccessibility was studied by Colle et al. (2010) and Lemmens et al. (2010) at different processing temperatures in tomato-based products and carrots. One study reported the effects of processing and lipid formulation on lycopene bioaccessibility. Under

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http://dx.doi.org/10.1016/j.foodchem.2016.04.037
0308-8146/

thermal treatment, lycopene bioaccessibility was significantly higher with 5% olive oil, than with the other types of oils tested, such as coconut or fish oil. (Colle et al., 2013). In addition to oil, fibers also influence the behavior of FSM during the cooking process and ultimately their bioaccessibility (Palafox-Carlos, Ayala-Zavala, & Gonzàlez-Aguilar, 2011). Complex emulsions with oil and fiber influence the FSM behavior and further detailed knowledge on the effect of thermal treatment of this food microstructure on the FSM behavior and bioaccessibility is currently missing. Moreover, few data are available on carotenoid bioaccessibility in complex matrices simulating ready-to-eat culinary preparation.

In this context of promoting traditional Mediterranean foods, our study focused on fat soluble micronutrient (FSM) behavior, during a traditional Tunisian cooking process and the first steps of digestion. The objective was to understand how this traditional processing method and specific formulation (high content in oil and fiber) impact FSM reactivity and bioaccessibility. Different carotenoid species (carotenes and xanthophyll) and α-tocopherol were studied in a complex emulsion matrix referring the traditional Tunisian dish called mloukhiya, which contains olive oil and *Corchorus olitorius*, (jute leaves also called Jew's mallow leaves) a green leafy hydrocolloid-rich vegetable.

2. Materials and methods

2.1. Sample preparation

This typical Mediterranean dish is prepared in Tunisia mainly by combining dried leaves of *Corchorus olitorius* L. ground in a very fine powder, tomato paste, water and olive oil and cooking the preparation. Other ingredients such as meat (rabbit, beef or mutton) or offals, green onions, spices (red pepper, curcuma) and salt are also used. The dish is cooked for 2–5 h. In order to gain greater insight into the effect of the cooking process, a simplified version of the recipe was prepared with the three main ingredients containing carotenoids and vitamin E (*Corchorus olitorius* L, olive oil, tomato paste) bought in a local market in Tunisia. Standard proportions of the main ingredients of the traditional Tunisian mloukhiya recipe were used: powder of *Corchorus olitorius* 11%, tomato paste 1%, olive oil 24%, and water 64%.

2.2. Laboratory processing

At time 0, *Corchorus olitorius* powder was mixed with olive oil and tomato paste into a homogeneous mixture. Then boiled water was added, the result was a hot, thick, highly mucilagenous broth. The mixture was heated under stirring (RW20-IKA Staufen, Germany) in an uncovered beaker for 2 h 30. A flow chart of the laboratory process is shown in Fig. 1. Water was added at 50 min (625 g) and at 100 min (400 g) of the cooking process to compensate for water evaporation. An Almemo probe 2690 (Ahlborn, Holzkirchen, Germany) with a standard K thermocouple was used to record the temperature during the process. The viscosity was measured with a HAAKE Vt550 viscometer (Thermo, Waltham, USA) with a geometry MV-DIN cell. The shear rate was set at 10 s^{-1} for 30 s, then increased at a constant rate of 1.9 s^{-1} min^{-1} until 200 s^{-1}. The dry matter content was measured gravimetrically in triplicate (24 h at 100 °C).

2.3. Carotenoid extraction of ingredients

Samples of ingredients, i.e. dried *Corchorus olitorius* (10 mg) or tomato paste (200 mg), were introduced in 15 ml tube with 1 ml of water, lysing matrix and a ceramic ball. Then 10 mL of ethanol/hexane (4:3, v/v) were added for extraction, followed by 40 s

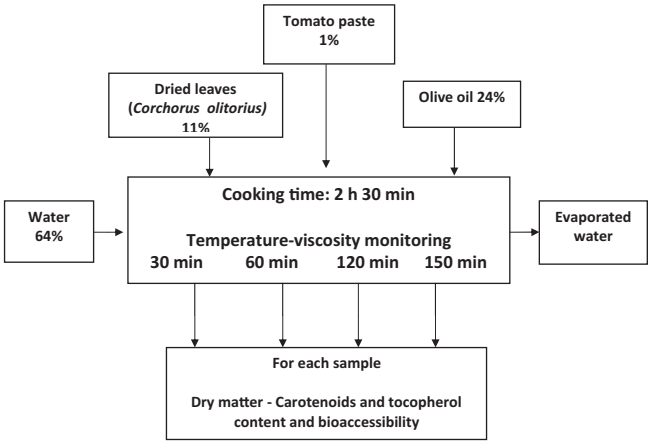

Fig. 1. Schematic overview of the experimental process.

full-speed high-energy (6 m s^{-1}) shaking using a FastPrep®24 homogenizer (MPBiomedicals, France). The mixture was then centrifuged (10,000*g*, 5 min). The upper organic phase was collected and the aqueous phase was again extracted with 5 ml hexane at least twice. The hexanic phases were pooled, dried with anhydrous sodium sulfate and evaporated under a vacuum centrifuge system (Genevac.EZ-2series, SP industries, UK.). The carotenoid extracts were redissolved in 300 μL of MTBE:methanol mixture (80:20, v/v) before HPLC injection.

2.4. Carotenoids and vitamin E extraction from the mloukhiya dish and olive oil

Carotenoids and tocopherols extraction was carried out according to a previously reported method (Messina et al., 2009). A 500 mg sample (olive oil or mloukhiya) was introduced in a 15 mL Pyrex tube and homogenized with 2 mL of ethanol containing 1% pyrogallol. The sample was then incubated for 2 min in a water bath at 70 °C. Saponification was performed for 30 min in a water bath at 70 °C by adding 1.5 mL of KOH (12 N). Samples were cooled and extracted twice with 5 mL of hexane after adding 2 mL of distilled water. Hexanic phases were evaporated under a vacuum centrifuge system (Genevac EZ-2series, SP industries, UK). The tocopherol extract was dissolved in 1 mL of ethanol before injection in an HPLC equipped with a fluorescence detector. The carotenoid extract was dissolved in 200 μL of MTBE/methanol mixture (80/20, v/v) before HPLC-DAD analysis .

2.5. Carotenoid extraction from digested mloukhiya samples

Carotenoid extraction was performed as previously described (Dhuique-Mayer et al., 2007). An aliquot of micellar aqueous or oil fraction from a digested sample (15 mL) was extracted 3 times with 10 mL of hexane and 5 mL of ethanol containing 100 μL of β-apo-8′-carotenal as internal standard. The pooled hexanic extracts were evaporated and redissolved in 100 μL of MTBE:methanol (80/20; v/v). The samples were injected according to analytical conditions described above.

2.6. HPLC analysis of carotenoids from the mloukhiya dish

Carotenoids were analyzed by reverse-phase-HPLC using an Agilent 1100 system (Massy, France), along a C_{30} column (250 × 4.6 mm i.d., 5 μm: YMC Europ GmbH, (Dinslaken Germany). The mobile phases were: H_2O as eluent A, methanol as eluent B and MTBE as eluent C at 1 mL min^{-1} flow rate. The column temperature