International Journal of Biological Macromolecules 111 (2018) 11–18

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Physicochemical, techno-functional, and antioxidant properties of a novel bacterial exopolysaccharide in cooked beef sausage

Imen Trabelsi [a], Naourez Ktari [b], Mehdi Triki [c], Intidhar Bkhairia [b], Sirine Ben Slima [a], Sameh Sassi Aydi [d], Samir Aydi [e], Asehraou Abdeslam [f], Riadh Ben Salah [a,*]

[a] Laboratory of Microorganisms and Biomolecules, Centre of Biotechnology of Sfax, Road of Sidi Mansour Km 6, B.P. 1177-3018, Sfax-Tunisia
[b] Laboratory of Enzyme Engineering and Microbiology, University of Sfax, National School of Engineering of Sfax (ENIS), B.P. 1173-3038 Sfax, Tunisia
[c] Chahia Company, Road of Gabes km 1, ZI Sidi Salem, 3002 Sfax, Tunisia
[d] Unit of Biodiversity and Valorization of Bioresources in Arid Zones, Faculty of Sciences of Gabes, University of Gabes, Tunisia
[e] Laboratory of Extremophilic Plants, Center of Biotechnology of Borj Cedria, BP 901, 2050 Hammam Lif, Tunisia
[f] Laboratory of Biochemistry and Biotechnology, Faculty of Sciences, University Mohammed Premier, Oujda, Morocco

ARTICLE INFO

Article history:
Received 23 October 2017
Received in revised form 20 December 2017
Accepted 23 December 2017
Available online 29 December 2017

Keywords:
Exopolysaccharide (EPS-Ca_6)
Physicochemical properties
Cooked beef sausages

ABSTRACT

This work investigates the effects of partial replacement of vitamin C (Vit C) with a purified exopolysaccharide (EPS-Ca_6) produced by *Lactobacillus* sp. Ca_6, on the antioxidant activities of cooked beef sausages during refrigerated storage. The physicochemical, techno-functional and viscosity properties of EPS-Ca_6 were also studied. Functional properties of EPS-Ca_6 were determined based on Water Holding Capacity (WHC), Oil Holding Capacity (OHC), emulsification activity, and foaming ability.

EPS-Ca_6 demonstrated excellent emulsifying and emulsion stabilizing properties. It was able to emulsify several food-grade oils and hydrophobic compounds, particularly corn oil and diesel with emulsification indexes of 90 and 100%, respectively at a concentration of 0.5%. The effect of EPS-Ca_6 on oxidative processes in cooked beef sausages during storage up to 12 days at 4 °C was evaluated. The obtained results showed a high rate ($p < 0.05$) of oxymyoglobin (OxyMb) and low lipid oxidation. Overall, our findings provided evidence that EPS-Ca_6 could be used as a natural additive for maintaining storage stability of cooked beef sausages, and could replace synthetic polymer in several industrial applications.

1. Introduction

Exopolysaccharides (EPS) are extracellular metabolites produced by several microorganisms, such as bacteria, fungi and blue-green algae. These microbial EPSs, have generally high molecular weight [1,2,3]. Among the wide variety of EPS-producing microorganisms, the Lactic Acid bacteria (LAB) are generally regarded as safe due to their wide use in food industry [2]. They are soluble in water and form a gel as food viscosity increases. Interestingly, EPSs synthesized by certain LAB including *Streptococcus*, *Lactobacillus*, *Lactococcus* and *Leuconostoc* have a variety of merits and thus attracted increasing interest through the years specially with the growing demand for natural polymers to various industrial applications [1,2]. Their end product is safe, natural and healthy, with improved texture and stability. In fact, these EPS remain stable in the gastrointestinal tract, enhancing probiotic bacteria colonization. Potential application in the improvement of the rheology, texture and mouthfeel of fermented milk products including yoghurt, cheese, viili and langfil [3] was also reported. They also exhibited high bioactive properties leading to potential pharmaceutical applications as antioxidants, antitumor, anti-inflammatory, antiviral agents, etc., urging many authors to study their structure and conformation [4]. The latter is important in order to determine the bioactive properties of these compounds [5].

In this context, we recently reported the purification of a new bacterial exopolysaccharides (EPS-Ca_6) produced by *Lactobacillus* sp.Ca_6 [6]. The EPS-Ca_6 showed a potential antioxidant activity *in vitro*, determined through DPPH scavenging activity, reducing power, β-carotene bleaching by linoleic acid assay, and metal chelating activities. It also exhibited antibacterial activity against pathogenic bacteria.

Because technological applications of natural polysaccharide depend on their functional properties besides their structural features, the presents study evaluates the viscosity, physicochemical and functional properties, especially emulsion formation of this EPS. In addition, for the study of its application in food product formulations, vitamine C was substituted with EPS-Ca_6 on cooked beef sausage. Its antioxidant activities were also determined against OxyMb and lipid oxidation during 12 days of storage at 4 °C.

* Corresponding author at: Laboratory of Microorganisms and Biomolecules, Centre of Biotechnology of Sfax, Road of Sidi Mansour Km 6, B.P. 1177-3018, Sfax-Tunisia
E-mail address: riadh.bensalah@cbs.rnrt.tn (R. Ben Salah).

https://doi.org/10.1016/j.ijbiomac.2017.12.127
0141-8130/

2. Materials and methods

2.1. Production and purification of the EPS-Ca_6

A novel microbial exopolysaccharide (EPS-Ca_6) was extracted and purified from *Lactobacillus* sp.Ca_6, and identified in our laboratory as described previously by Trabelsi et al. [7].

2.2. Physico-chemical analysis

Moisture and ash contents of EP-Ca_6 were determined according to the AOAC methods number 930.15 and 942.05, respectively [8]. Protein contents were analyzed by the Kjeldahl method according to standard method 984.13 and estimated by multiplying total nitrogen content by 6.25 factors [8]. Crude fat was determined gravimetrically after Soxhlet extraction of dried samples with hexane. Total carbohydrates were determined by the phenol-sulphuric acid method [9].

pH (1% solution at 25 °C) was measured using a digital pH meter (Systronics Instruments, India) with the glass electrode completely immerged into the solution.

Viscosity measurements at various concentrations in H_2O (2, 2.5, and 3 g/l) of EPS-Ca_6 were determined at 25 °C by means of a digital viscometer (NDJ-1, Japon) at 30 rpm spindle rotation with rotor N° 4.

The morphology of EPS-Ca_6 was determined using an optical microscope (Nikon Eclipse E400, Kanagawa, Japan) equipped with a digital camera and image processing software Lucia G (Nikon, Japan), the powder sample images were obtained in the analyzing and polarizing mode.

EPS-Ca_6 color was determined using a Color Flex spectrocolorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) and reported as L*, a* and b* values.

2.3. Determination of molecular weight

The Molecular weight of the EPS-Ca_6 was determined according to the method reported by Bayar et al. [10]. Briefly, 2 mg EPS were dissolved in 2 mL distilled water, using a gel filtration high pressure chromatography equipped with a Zorbax PSM 300 column (6.2 * 250) refractive index detector with a mobile phase of bi-distilled water, a flow rate of 0.8 ml/min and 30 °C. 100 μl of polysaccharide solution were injected after a filtration through a 0.2 μm membrane.

2.4. UV absorption peak detection

EPS-Ca_6 was dissolved in distilled water to a final concentration of 0.1%. The UV absorption spectrum of the sample was recorded in the wavelength range of 200–800 nm [11].

2.5. X-ray diffraction (XRD)

The X-ray diffractograms of EPS-Ca_6 were obtained at room temperature on an X-ray diffractometer (D8advance, Bruker, Germany). The patterns were collected in the 2θ ranges 5–80° with a step size of 0.05° and a counting time of 5 s/step.

2.6. Functional properties

2.6.1. Water-holding (WHC) and oil-holding (OHC) capacities

WHC and OHC were measured as previously described by Lin, et al. [12]. EPS-Ca_6 (100 mg) was dispersed in 50 ml of distilled water or 10 ml of soybean oil to determine WHC and OHC, respectively. The ratio between the weight of the tube content after draining and the weight of the lyophilized EPS-Ca_6 was determined and the capacity (%) was reported as grams of water or oil bound per gram of the EPS-Ca_6 on a dry basis.

2.6.2. Emulsifying activities

2.6.2.1. Preparation of emulsions. The emulsifying activities of EPS-Ca_6 were assayed as reported by Freitas et al. [5]. After 1, 24 and 168 h, respectively, emulsification indices E_1, E_{24} and E_{168}, were calculated as follows:

$$Et = he/ht \times 100$$

where *he* (mm) is the emulsion layer height and *ht*. (mm) is the mixture overall height after t hours. *he* and *ht*. were measured using a ruler.

2.6.2.2. Determination of emulsifying activities of EPS-Ca_6 with different oils and hydrocarbons. The emulsifying activities and the stabilizing capacity of EPS-Ca_6 solution (0.5%) were performed against different hydrophobic compounds including oils (corn oil and olive oil) and hydrocarbons (diesel, chloroform, toluene, and n-hexane). Based on E_1, E_{24}, and E_{168}, the oil and hydrocarbon which yielded the most stable emulsion were selected for further study.

2.6.2.3. Effect of EPS-Ca_6 concentration on emulsifying activity. Olive oil and diesel were selected as the best oil and hydrocarbon, respectively, and hence they were chosen for subsequent assays.

The emulsions prepared with these compounds were studied with respect to different EPS-Ca_6 concentrations ranging from 0.1 to 10%, using the emulsion indexes E1, E24, and E168.

2.6.2.4. Effects of pH, temperature and ionic strength on emulsion stability. The EPS-Ca_6 (0.5%) emulsion stabilizing capacity was evaluated at a large range of pH (2.0–12.0), temperature (20–100 °C), and ionic strength (0.2–2.0 M NaCl). To study the thermal stability, emulsions were placed at 20, 40, 60, 80, and 100 °C during 1 h. After slow cooling to room temperature, emulsions were left for 24 h to calculate E_{24}.

2.6.3. Foaming properties

The Foam capacity (FC) and stability (FS) of EPS-Ca_6 were studied according to the method of Bayar et al. [10].

2.7. Effect of EPS-Ca_6 on cooked beef sausage oxidation

2.7.1. Cooked beef sausage product preparation

EPS-Ca_6 was assessed for its lipid antioxidant activity in cooked beef sausage. The standard sausage (FC) formulation consisted of: meat (65%), cold water (23%), modified starch (8.45%), NaCl (1.3%), $NaNO_2$ (0.047%), sodium tripolyphosphate (0.31%), carrageenan (0.7%), and Vit C (0.125%). The same ingredients were used in the different cooked beef sausage formulations, expect for Vit C which was substituted by the EPS-Ca_6. Meat and ingredients were obtained from a local meat company (Chahia, Sfax-Tunisia). Four formulations were prepared:

FC (Control): sausage with Vit C.
F1: sausage without Vit C or EPS-Ca_6.
F2: sausage with Vit C at 0.0625% and EPS-Ca_6 at 0.0625%.
F3: sausage with EPS-Ca_6 at 0.125%.

Dry ingredients were slowly added to the ground meat as powders during processing. Then, cold water was added. Stuffing was carried out manually into 27-mm-diameter reconstituted collagen casings and hand-linked to form approximately 8-cm-long links. Subsequently, sausages were heat-processed in a temperature controlled water-bath (Haake, Kalsruhe, Germany) maintained at 90 °C until a final internal temperature of 74 °C was reached. Afterwards, sausages were cooled immediately and stored at 4 °C until analysis.