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Research Paper

Nucleic acids from agro-industrial wastes: A green recovery method for fire retardant applications

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ABSTRACT

Two different agro-industrial biological wastes, namely spent brewer's yeast and vegetable scraps have been considered as inexpensive sources of nucleic acids (NAs) to be employed for conferring flame retardant features to cotton fabrics. A simple, cheap and green extraction method has been set-up for each matrix. The yields, purity grades and molecular sizes have been assessed and compared with two different commercially available purified DNA. The developed extraction procedures have shown a high level of reliability for the recovery of nucleic acids from both agro-industrial wastes. The results of the flammability tests with the extracted NAs clearly indicated that, the add-on value on the fabrics being equal, the NAs extracted from spent brewer's yeast cells are able to provide self-extinction to cotton and reproduce the fire behaviour obtained with highly expensive commercially purified DNAs. Thus, the proposed procedures may be applied for large-scale NA recovery from inexpensive agro-food wastes to obtain partially purified NA suitable for fire retardant applications. Moreover, the developed extraction procedures can be considered an addition to the growing body of more rational waste valorisation techniques within the bio-refinery approach and circular economy concept.

1. Introduction

At present, agri-food waste and by-product valorization processes are focused on the recovery of low-value compounds such as fertilizers and building blocks, which can be used directly or as carbon sources in fermentation processes and for energy production (i.e. biogas). Some of these applications have been optimized over the last few decades, while other applications are still at their early stages of development. As far as the biomacromolecules from wastes are concerned, although the recovery of polysaccharides, proteins and lipids is a well-known process (Liguori and Faraco, 2016; Mohan et al., 2016), the nucleic acids (NAs) fraction has not been considered within a bio-refinery approach.

In the last five years, the scientific literature on textile flame retardants (FRs) has clearly indicated that new radical solutions are necessary, and efficient and environmentally friendly products have to be found in order to replace the "traditional" FRs that, despite their good efficiency, may suffer from some environmental drawbacks. One of the latest approaches, which is worth of further investigation, is that of the use of biomacromolecules, such as proteins and DNA, as FRs, thanks to their chemical structures and composition. In fact, some of them have already been proven to be very effective at a lab-scale, exploiting green finishing processes (i.e. by applying aqueous solutions/dispersions directly to different textiles, using traditional processes, such as impregnation/exhaustion and spraying). Recently, low molecular weight DNA was employed as an effective intumescent flame retardant coating for cotton fabrics, since it has demonstrated to provide self-extinction in horizontal flame spread tests and a substantial reduction in the heat release rate in cone calorimetry tests (Alongi et al., 2013; Bosco et al., 2015). The DNA's fire retardant activity has been ascribed to the simultaneous presence of phosphate groups, nitrogen bases and deoxyribose sugars in the same molecule, which makes the biomacromolecule to be considered as an intumescent-like product. On the other hand, proteins derived from milk, such as caseins and whey proteins, show a fire retardant effect when deposited on cotton fabrics. Although they cannot provide self-extinction of the flame, they are able to significantly reduce the burning rate and increase the final residue (Alongi et al., 2014; Bosco et al., 2013).

On the basis of these findings, it is possible to consider that DNA could be a valid alternative to traditional halogenated FRs, and could offer the opportunity of opening a new research scenario related to this unconventional application. However, the use of commercially available products might not be suitable because these chemicals mainly

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used in molecular biology for analytical purposes (Dellaporta et al., 1983), are available in very small quantities and are extremely expensive.

For this reason, valid DNA sources have to be found, and environmentally friendly and cost-effective recovery techniques have to be developed. Bearing in mind that the amount of extracted biomacromolecules has to be sufficient to treat large fabric surfaces, it seems logical that the available DNA sources that are inexpensive and accessible in large quantities should be considered.

In this context, wastes from agro-food industries that process biomass or food may represent good sources of nucleic acids that meet all of the above-mentioned requirements.

The traditional techniques that are usually applied in molecular biology and medical research fields cannot be exploited for the extraction of NAs from these bio-wastes. In fact, conventional techniques are suitable for the treatment of very small starting samples in the order of mg, and consequently the obtainable quantity of nucleic acids is very limited, i.e. of the order of μg. In addition, these techniques utilize a sequence of numerous separation and purification steps, which makes them time consuming. Finally, they can be expensive because the high grade of DNA or RNA purification requires highly pure, expensive and, sometimes, even toxic reagents, like phenol and chloroform (Dellaporta et al., 1983; Guillemaut and Marechal-Drouard, 1992; Lõoke et al., 2011).

In the present work, two different food industry wastes have been investigated as potential nucleic acids sources, namely spent brewer's yeasts and mixed vegetable scraps from a processing plant that handles vegetables and fruit to obtain IV range products. Brewer's yeast traditionally belongs to *Saccharomyces* spp; spent yeast is one of the main by-products of beer production, and it is mainly exploited in animal feed. Other applications, such as a substrate in microbial cultures or for the extraction of cell components, (e.g. β-glucans, proteins, B vitamins and minerals), can also be mentioned (Suphantharika et al., 2003; Podpora et al., 2015). Vegetable wastes from food industry plants are discharged in remarkable quantities. The quantities and compositions of these wastes depend, to a great extent, upon the seasonal periodicity of the agricultural productions. They are mainly utilized for the production of compost, livestock feeding and fermentation for biogas or ethanol production (Di Donato et al., 2014; Nagarajan et al., 2014; Bouallagui et al., 2005).

An attempt has been made in the present work to develop and optimize simple, sustainable and cost-effective methods in order to extract NAs with high yields, using considerable quantities of spent brewer's yeasts or mixed vegetable wastes. Because of the different biological origins of the two wastes (vegetal tissue or microorganism), two different specific recovery methods were developed. The obtained low purified NA extracts have been applied to cotton fabrics and tested to assess their flame retardant properties in terms of horizontal flame spread propagation.

2. Materials and methods

2.1. Materials

The mixed vegetable wastes were supplied by a food processing plant (Stroppiana Ortofrutticola S.p.A. Chieri, Turin, Italy) and were derived from a IV range vegetable production line. Because of the seasonal variability of the agricultural cultivations, the batches, which arrived at the laboratory at different times, were heterogeneous in composition. Almost all of the batches consisted of a portion of leafy vegetables (lettuce, chard, celery, cabbage leaves, red chicory, rocket salad leaves) and another made up of small pieces of turnip, onions, peppers, courgettes, potatoes, carrots and pumpkin. The vegetable matrices considered have been indicated as follows: mixed leafy vegetables (MLV) and leafy vegetables (LV). MLV mainly comprised leafy vegetables and a small portion of vegetable pieces (about 10%), while LV matrix was only composed of leafy vegetables. The matrices were separated into small portions (500 g) and stored at +4 °C (for a maximum of 48 h) or at −20 °C before use.

Spent brewer's yeast was kindly supplied by Heineken Italia S.p.A. (Aosta, Italy) and Birrificio Della Granda (Cuneo, Italy) and stored at +4 °C until it was used.

Cotton fabrics (COT, 200 g/m^2) were purchased from Fratelli Ballesio S.r.l. (Torino, Italy) and cut into 100 × 100 mm^2 square specimens.

A liquid commercially available detergent (containing 5–15% anionic surfactants) was used. Commercial DNA from herring testes and herring sperm, NaCl, ethanol (at 99.8% purity), Tris (hydroxymethyl) aminomethane Hydrochloride (Tris-HCl) (pH 8) and Ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich S.r.l. Milano, Italy and used as received.

2.2. NA extraction from the mixed vegetables

First, mixed vegetable wastes stored at −20 °C were thawed. The vegetable portions (30 g) were then ground using a mortar and pestle or a hand blender and were then mixed with the extraction buffer (liquid detergent 10% v/v, NaCl, 10% w/v). The extraction buffer:vegetable dry weight ratio was set at 1:7. The extraction was carried out at 65 °C for 30 min. Then, the waste was separated from the aqueous extract through filtration, using a nylon gauze, and then using qualitative filter paper. Cold ethanol (stored at −20 °C) was then added to the aqueous fraction in a 1:1 (v:v) ratio. Precipitation was first performed overnight at −20 °C. The alcoholic upper phase, containing the precipitated nucleic acids, was recovered and centrifuged at 4100 rpm for 10 min. Finally, the precipitated pellet, containing NAs, was re-suspended with distilled water.

Different parameters were changed, one at a time, to optimize the extraction procedure, as follows: i) the starting material was either stored at −4 °C or at −20 °C before extraction; ii) 99.8% ethanol was further diluted with bi-distilled water to 96 and 90% purity grade and used for the precipitation step; iii) the precipitation was carried out at two different temperatures (+4 and −20 °C); iv) the precipitation was performed at −20 °C for different incubation times, namely 1, 2, 3, 4, 8 h and overnight.

2.3. NAs extraction from the spent brewer's yeast

Beer slurries were stored in two different ways before NAs extraction: one batch was maintained at +4 °C for more than 6 months, and, just before extraction, the yeast cells were centrifuged and washed three times with bi-distilled water (AY); in a second batch, the cells were immediately centrifuged, washed three times with bi-distilled water and the pellet stored at −20 °C until they were used (FY).

Each of the extraction buffers listed hereafter was added at a ratio of 1 mL per 8 mg of the yeast pellet weight. The mixture was frozen at −20 °C for 24 h and then thawed three times at 65 °C for 15 min. After thawing, cells were mechanically disrupted using 1 and 2 mm diameter glass beads (1 mg:5 g yeast:glass beads ratio) and the mixtures were then subjected to ball milling for 10 min. Glass beads were then recovered and cell debris removed by means of centrifugation (4100 rpm for 10 min). Further chemical purification steps were avoided. However, physical purification was carried out through different types of filtration: microfiltration (porosity 0.7 μm) and ultrafiltration (cut-off of 10 kDa).

Nucleic acid recovery from yeast was optimized by changing some parameters, one at a time, as follows:

- different extraction buffers were tested, namely:
- TrisHCl50 mM + NaCl 50 mM + EDTA10 mM, pH 8 (labelled TNE 50/50/10)