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Seaweed extracts as potential functional ingredients in yogurt

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ABSTRACT

Yogurt was manufactured containing extracts (0.25% and 0.5% (w/w)) prepared from *Ascophyllum nodosum* (100% H_2O (AN_{100}), 80% ethanol:20% H_2O (AN_{80e})) and *Fucus vesiculosus* (60% ethanol:40% H_2O (FV_{60e})). Yogurt composition, shelf-life parameters, stability and bioactivity of seaweed extracts in yogurt was examined over 28 days. Yellowness 'b*' was higher ($P < 0.05$) in yogurts containing FV_{60e} and AN_{80e}. Yogurts containing AN_{80e} (0.5%) and FV_{60e} (0.5%) had lower ($P < 0.05$) levels of lipid oxidation. The pH, microbiology and whey separation in yogurt were unaffected by seaweed extract addition. Yogurt modulus was higher in control yogurts. Control and AN_{100} (0.25% and 0.5%) yogurts were most accepted by sensory panellists. Antioxidant activity (DPPH) of seaweed extracts in yogurt was stable as a function of storage time. Yogurt and digestates did not affect the antioxidant status (CAT, SOD and GSH assays) or protect against H_2O_2-induced DNA damage in Caco-2 cells.
Industrial relevance: The research work and results presented in this manuscript are of high industrial importance.
Yogurt and related products are some of the most commonly manufactured and consumed food products worldwide. In recent years yogurt and other dairy products have been used as carriers for functional bioactive food ingredients, or nutraceuticals. Seaweeds contain a range of bioactive compounds with reported health benefits and represent a potentially exploitable source of functional ingredients for the dairy industry. This manuscript demonstrates that extracts from *Ascophyllum nodosum* can be incorporated successfully into a fermented dairy product such as yogurt.

1. Introduction

Yogurt and related products are some of the world's most commonly manufactured and consumed food products. From a nutritional perspective, yogurt is widely perceived as a healthy food as it contains protein, riboflavin, vitamins B_6 and B_{12}, and calcium. Additionally, in recent years yogurt and other dairy products have been used as carriers for functional food ingredients, or nutraceuticals. Nutraceuticals are defined as food components which demonstrate physiological benefits or reduced risk of chronic disease beyond their basic nutritional function (Shah, 2001).

Bifidobacterium and *Lactobacillus*-enriched probiotic yogurt is among the most common type of functional food products marketed worldwide. Yogurt is also used commercially as a carrier for gut-friendly prebiotics (Thomas & Greer, 2010; Mishra Pandey & Mishra, 2015) and cholesterol lowering phytosterols (Moreau, 2004). Laboratory scale studies have investigated yogurt as a possible carrier for other functional food ingredients such as omega-3 fatty acids, vitamins and minerals (Hekmat & McMahon, 1997; Achanta, Aryana, & Boeneke, 2007; Sabeena Farvin, Baron, Nielsen, & Jacobsen, 2010).

Antioxidants such as polyphenols and carotenoids are popular functional ingredients which exhibit a range of health benefits including *anti*-cancer, eye protective, heart protective and anti-diabetic properties (Pandey & Rizvi, 2009). Studies have investigated yogurt as a potential carrier for antioxidant compounds with the aim of increasing the health benefits and quality attributes of the resulting product. Cossu, Juliano, Pisu, and Alamanni (2009) produced yogurts enriched with crude extracts from artichoke (*Cynara scolymus* L.), strawberry-tree fruit (*Arbutus unedo* L.) and cherry (*Prunus avium* L.) and found that the fortified yogurts exhibited significantly higher antioxidant activity (total phenol content (TPC), FRAP and DPPH scavenging activities) compared to the non-enriched yogurt. Similarly, green and Pu-erh tea infusions (5–15%) increased the antioxidant capacity (FRAP and DPPH scavenging activities) of yogurt (Najgebauer-Lejko, Sady, Grega, & Walczycka, 2011).

Brown seaweeds such as *Ascophyllum nodosum* and *Fucus vesiculosus* contain a range of proteins, fats, carbohydrates, minerals, vitamins and bioactive compounds (Miyashita, Mikami, & Hosokawa, 2013).

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Polyphenolic compounds previously quantified in brown seaweed include caffeic acid, chlorogenic acid, coumaric acid and catechins (Keyrouz et al., 2011). Phlorotannins, polyphenolics unique to marine algae, are found at the highest levels (2–10%) in brown algal species (Ragan & Glombitza, 1986). Due to the complexity and wide diversity of compounds present in seaweeds, characterisation and quantification of target compounds is difficult. *In vitro* antioxidant assays (*e.g.* total phenol content (TPC), FRAP and DPPH radical scavenging activities) are frequently used to assess the antioxidant activity and potency of seaweed extracts (Zaragozá et al., 2008).

Polyphenol rich seaweed extracts are commonly prepared using polar solvents such as water and/or alcohol and represent a concentrated form of bioactive compounds (*e.g.* antioxidants) present in seaweed. Such extracts are a potentially unique source of functional food ingredients. To date, the scientific literature contains limited data on the toxicological evaluation of seaweed extracts. However, Zaragozá et al. (2008) reported non-toxic effects of *Fucus vesiculosus* extracts *in vivo*.

A number of seaweed and seaweed-derived extracts have demonstrated superior antioxidant activity compared to terrestrial plants (Budhiyanti, Raharjo, Marseno, & Lelana, 2011). O'Sullivan et al. (2011) demonstrated that crude water-methanol prepared extracts from a range of brown seaweeds exhibited *in vitro* antioxidant activity and DNA protective effects against H_2O_2-induced DNA damage in Caco-2 cells. Similar findings were reported for water and aqueous ethanol extracts of *Ascophyllum nodosum* and *Fucus vesiculosus* (O'Sullivan et al., 2013). In the scientific literature, studies investigating the addition of seaweed extracts to dairy products are limited. A recent study by our research group examined the potential of seaweed extracts as functional antioxidant ingredients in milk (O'Sullivan et al., 2014). The behaviour of such extracts in a fermented dairy product such as yogurt is unknown and merits investigation.

The objective of this study was to manufacture yogurt containing three seaweed extracts (*Ascophyllum nodosum*: 100% H_2O (AN_{100}), 80% ethanol:20% H_2O (AN_{80e}) and *Fucus vesiculosus*: 60% ethanol:40% H_2O (FV_{60e})). The effect of seaweed extracts (0.25 and 0.5%) (*w*/w) on the quality and shelf-life (colour, lipid oxidation, pH, microbiology, whey separation, rheology, and sensory analysis) of yogurts was investigated over a 28 day storage period. The stability of seaweed extracts in yogurt was assessed using the DPPH radical scavenging activity assay. Seaweed extract enriched yogurts were subjected to an *in-vitro* digestion procedure to assess the antioxidant capacity of the yogurts before and after digestion. Antioxidant activity of the yogurt and yogurt digestates was determined using *in-vitro* antioxidant (DPPH radical scavenging activity and ferrous-chelating activity (FICA)) and cellular antioxidant (catalase (CAT), superoxide dismutases (SOD) and glutathione (GSH)) assays. The ability of yogurt and yogurt digestates to protect against oxidant-induced DNA damage in human adenocarcinoma Caco-2 cells was also investigated.

2. Materials and methods

2.1. Materials

Fresh whole milk was obtained from a local retail outlet. Yogurt culture (Yo-flex) was obtained from CHR Hansen (Cork). Cell culture materials were as described in O'Sullivan et al. (2013). Agar was purchased from Oxoid Ltd., Basingstoke, Hampshire, England.

2.2. Addition of seaweed extracts to milk and yogurt manufacture

Ascophyllum nodosum was collected from New Quay, Co. Clare, Ireland and *Fucus vesiculosus* was harvested from Spiddal, Co. Galway, Ireland and transported to the laboratory in the National University of Ireland, Galway (NUIG) for taxonomic identification, washing and storage at −20 °C. Seaweed samples were supplied to the Teagasc Food Research Centre (Ashtown, Dublin 15, Ireland) for seaweed extract manufacture. Seaweeds were freeze-dried at −20 °C for 72 h, vacuum-packed and stored at −80 °C prior to extraction.

Seaweed extracts (*Ascophyllum nodosum*: 100% H_2O (AN_{100}), 80% ethanol:20% H_2O (AN_{80e}) and *Fucus vesiculosus*: 60% ethanol:40% H_2O (FV_{60e})) were manufactured and characterised (total phenol content (TPC) - $FV_{60e} > AN_{80e} > AN_{100}$ ($P < 0.05$); DPPH radical scavenging ($AN_{100} = AN_{80e} = FV_{60e}$) and ferrous-ion chelating activity (FICA - $AN_{100} = AN_{80e} > FV_{60e}$ ($P < 0.05$) assays) as described in O'Sullivan et al. (2014). Extracts (AN_{100}, AN_{80e} and FV_{60e}) were added to milk (1000 ml) at concentrations of 0.25% and 0.5% (*w*/w) and mixed for 5 h at 4 °C with a magnetic stirrer to aid dissolution. Seaweed extract-containing milk was heated in a waterbath until 93 °C was reached and then held at this temperature for 15 min and subsequently cooled to 43 °C. Yogurt culture (Yo-flex, CHR Hansen) was added at a concentration of 0.1% (*v*/v) and milk samples were further incubated at 43 °C until a pH of 4.5 was reached. Yogurt samples were subsequently stirred and ~95 g portions were packaged aseptically in 100 ml sterile containers (Sarstedt Ltd., Co. Wexford, Ireland) and stored for 28 days at 4 °C. Quality and shelf-life measurements (colour, lipid oxidation, pH, microbiology, whey separation, rheology, and sensory analysis) were recorded at 7 day intervals up to 28 days of storage.

2.3. Compositional analysis

The moisture and fat content of yogurt were measured using the SMART Trac rapid moisture/fat analyser (CEM Corporation, NC, USA). Protein nitrogen (×6.38) was determined by the Kjeldahl method of the Association of Official Analytical Chemists (AOAC, 1995). The ash content was determined using a muffle furnace (AOAC, 1995). The carbohydrate content was calculated by difference. The composition of commercially available natural yogurt was also analyzed for comparative purposes. Compositional analysis results were expressed as percentage values, %.

2.4. Surface colour measurement of yogurt

The surface colour of yogurt samples was measured by Minolta colorimetry as described in O'Sullivan et al. (2014).

2.5. Measurement of lipid oxidation in yogurt

Lipid oxidation in yogurt was measured following the procedure of Fenaille, Mottier, Turesky, Ali, and Guy (2001). Results were expressed as 2-thiobarbituric acid-reactive substances (TBARS) in mg malondialdehyde (MDA)/kg yogurt.

2.6. pH and microbiology of yogurt

Yogurt pH was measured using a pH meter (Seven Easy portable, Mettler-Toledo GmbH, Switzerland) by directly inserting the pH probe into the yogurt samples. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* counts were determined in yogurt using the pour plate technique with M17 agar (supplemented with sterile lactose solution (10% w/v)) (Oxoid Ltd.) and MRSA (de Man, Rogosa and Sharpe agar), respectively. The MRSA plates were placed in heat-sealed bags in the presence of Anaerocult® A (Merck Millipore, Germany). The MRSA and M17 plates were incubated at 37 °C for 4 days. Results were expressed as $\log_{10}$CFU (colony forming units)/g yogurt.

2.7. Whey separation in yogurt

Whey separation in yogurt was assessed according to the method of Keogh and O'Kennedy (1998) with modifactions. The % whey separation was measured as follows: