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Hybrid carrageenans as beer wort fining agents

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ABSTRACT

Pure Na^+ forms of furcellaran (β/κ-hybrid carrageenan), κ-carrageenan, κ/ι-hybrid carrageenan, ι-carrageenan and a hybrid carrageenan mix (made up of κ-, ι-, λ- and ν-carrageenans) were tested as beer wort fining agents. To find out which of the used carrageenans are the best beer wort clarifiers and whether they are prone to overdosage, polysaccharides with varying concentrations and molecular weights were tested. The polysaccharides were characterized by ^{1}H NMR spectroscopy and size-exclusion chromatography. The fining efficiency of the tested polysaccharides was measured nephelometrically. Ultrasonicated and alkali treated carrageenan preparations were tested as beer wort fining agents and compared to the native samples. Undegraded furcellaran, κ-carrageenan and κ/ι-hybrids were efficient in lowering the turbidity of various beer worts, but the addition of ι-carrageenan and the carrageenan mix caused the turbidity to rise above the unfined sample value. Compared to the degraded samples, native galactans were more effective in lowering the beer wort turbidity. Higher concentrations of ultrasonicated carrageenans are needed to clarify the beer wort to similar turbidity values. Alkali treated carrageenans were more efficient beer wort fining agents compared to the native samples.

1. Introduction

Carrageenans are a class of linear, highly sulfated galactans (Fig 1) extracted from various red algal species which are utilized for their stabilizing, thickening and gelling properties (Barabanova, et al., 2013; van de Velde, 2008). The polysaccharides are made up of alternating 3-linked-β-D-galactopyranoses (G units) and 4-linked-α-D-galactopyranoses (D units) (Knutsen, Myslabodski, Larsen, & Usov, 1994; Usov, 1992). The disaccharide repeating moieties (carrabiose units) are classified according to the number and the position of the ester sulfate groups (S) and by the presence of the 3,6-anhydro-bridge (DA unit) of α-linked D-galactose residues. The β-linked G units occur as 2-, 4- or 6-sulfate, or are sometimes unsulfated, the α-linked anhydro units occur as unsulfated, as 3,6-anhydro-2-sulfates, as 2-, or 6-sulfates or as 2,6-disulfates. The hydroxyl groups can be further substituted by methoxyl, pyruvate ketal or branched glycoside groups (Barabanova, et al., 2013; Usov, 1992). Currently there are about 20 different principal carrageenan types recognized, which are referred to by Greek letters (Barabanova, et al., 2013).

The most commonly used sulfated galactans are κ-, ι-, λ-carrageenans which in their idealized structure have one (G4S-DA), two (G4S-DA2S) and three sulfate (G2S-D2S,6S) groups per carrabiose unit (Anderson, Dolan, & Rees, 1973; Knutsen, et al., 1994). Carrageenans however are often a mixture of heterologous polysaccharides (known as hybrids) instead of a pure form of a sole carrageenan type (Barabanova, et al., 2013; Dyrby, et al., 2004; Therkelsen, 1993; Usov, 1992; van de Velde, Peppelman, Rollema, & Tromp, 2001). The term 'repeating unit' refers to the principal disaccharide unit that occurs in the carrageenan structure (Therkelsen, 1993). An example of a hybrid carrageenan is furcellaran (made up of β-, κ-carrageenans), which is similar to κ-carrageenan – both have 3,6-anhydro-D-galactose in their structure, but the 3-linked galactose units in β-carrageenan lack the sulfate group (Anastyuk, et al., 2011; Barabanova, et al., 2005; Correc, et al., 2012; Renn, et al., 1993). The best known hybrid carrageenan is probably the kappa-2 – also known as κ/ι-hybrid or weak (gelling) kappa-carrageenan (Azevedo, et al., 2013; van de Velde, et al., 2005; van de Velde, et al., 2001; Villanueva, Mendoza, Rodrigueza, Romero, & Montaño, 2004).

Wort is the liquid extracted during the mashing process in early stages of beer brewing (Trantham, 2017). It consists of sugars that will be fermented by the brewing yeast to produce alcohol. The sugars are usually extracted from dried, malted barley although wheat, corn or rye may also be included (Krottenthaler, Back, & Zarnkow, 2006; Ryder & Power, 2006; Trantham, 2017; Willaert,

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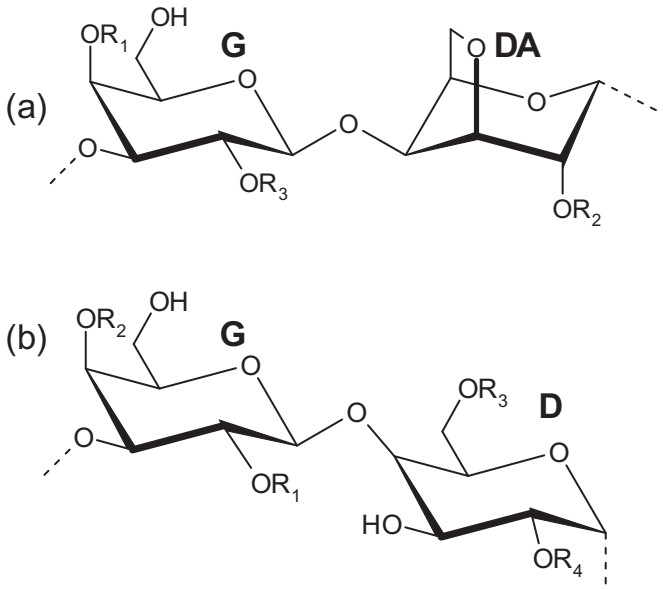

Fig. 1. Idealized structures of some carrageenans. (a) β-carrageenan ($R_1 = R_2 = R_3 = H$), κ-carrageenan ($R_1 = SO_3^-$, $R_2 = R_3 = H$), θ-carrageenan ($R_2 = R_3 = SO_3^-$, $R_1 = H$) and ι-carrageenan ($R_1 = R_2 = SO_3^-$, $R_3 = H$); (b) μ-carrageenan ($R_2 = R_3 = SO_3^-$, $R_1 = R_4 = H$), λ carrageenan ($R_1 = R_3 = R_4 = SO_3^-$, $R_2 = H$), ν-carrageenan ($R_2 = R_3 = R_4 = SO_3^-$, $R_1 = H$).

2007). The mashing takes about 90 min during which the barley's enzymes hydrolyse starch and other polysaccharides to fermentable sugars, primarily maltose and maltotriose (Evans, van Wegen, Ma, & Eglington, 2003). Large proteins are also hydrolysed to polypeptides and amino acids during mashing, which the yeast cells use in the later stages of beer brewing (Osman, Coverdale, Onley-Watson, Bell, & Healy, 2003). After mashing the grains are removed and the solution is brought to a boil, which takes a further ~90 min. That sterilizes the wort and at the same time the flavour and stabilisation providing hops are added. Boiling denatures the proteins extracted from the grains, causing them to coagulate and form large flocs which precipitate – a process termed 'hot break'. During boiling polyphenols are also extracted from the grains and hops which bind to proteins not precipitated during boiling and will cause a precipitating complex and/or haze at low temperatures (a process termed 'cold break') in the beer later on – an effect termed 'chill haze'. The proteins will resuspend into solution once the wort/beer is warmed up to room temperature. The total precipitation is aided by high pH of the wort (a typical wort pH ranges from 5.3–5.7; the most efficient pH-value for wort fining with carrageenans is 5.1–5.3) and longer boiling times, but is mainly dependent on the type and amount of grains used (Dale, Morris, & Lyddiatt, 1995; Hough, Stevens, & Young, 1982). Fining agents (copper finings, kettle finings) are added to the wort ~10 min before the end of the boil to speed up the aggregation and sedimentation of the protein-protein and protein-polyphenol complexes during cooling. The exact fining mechanism is unclear, but it is thought that negatively charged carrageenans interact with positively charged proteins, forming an insoluble complex which precipitates out of the wort. Transition in carrageenan chain conformation (influenced by the solution pH-value) during cooling is also important (Dale, Morris, & Lyddiatt, 1995; Dale, Tran, & Lyddiatt, 1996a). Correct fining agent dosage (Fig 2) improves beer filtration throughput, provides higher colloidal stability and a more compact sedimentation, which leads to reduced wort losses (larger amounts of clarified wort can be fermented and beer produced), shortened boiling time and a clearer beer with a longer shelf-life (Dale, Morris, & Lyddiatt, 1995; Trantham, 2017). The most widely

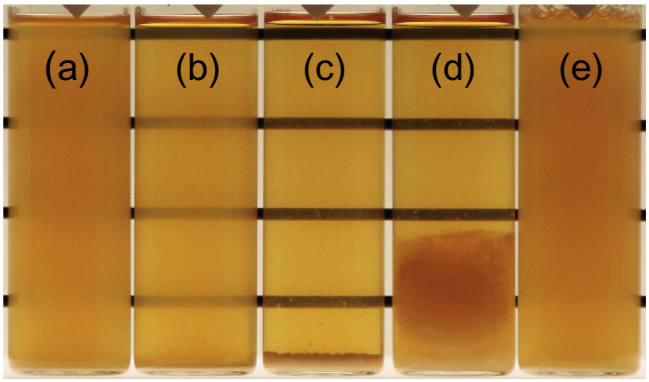

Fig. 2. Comparison of fining agents tested on Château Pale ale beer wort. (a) Blank, (b) underdosage (2.5 ppm of furcellaran), (c) correct dosage (10 ppm of furcellaran), (d) overdosage (40 ppm of κ-carrageenan), (e) incorrect fining agent (20 ppm of ι-carrageenan). The photograph was taken against a background of horizontal stripes.

used fining agents are κ-carrageenans (in either pure form or as a semi-refined product) and the dosage ranges between 1–6 g/hL (10–60 ppm) depending on the wort type (Dale, Morris, & Lyddiatt, 1995; Dale, Tran, & Lyddiatt, 1996a, 1996b; Leather, 1998; Poreda, Zdaniewicz, Sterczynska, Jakubowski, & Puchalski, 2015).

There have been studies dedicated to understanding the mechanism behind carrageenans ability to clarify wort (Dale, Morris, & Lyddiatt, 1995; Dale, Tran, & Lyddiatt, 1996a; Dale, Tran, & Lyddiatt, 1996b), but unfortunately there is no data about structures (content of 3,6-anhydrogalactose and/or precursors) and molecular weight distributions of carrageenan samples used. Furthermore there has not been an extensive study on hybrid carrageenan mediated beer wort clarification and it is unclear how the wort turbidity is affected by the addition of alkali treated carrageenan samples (alkali treatment cyclizes the structure, resulting in a higher 3,6-anhydrogalactose content and a more homogenous preparation). The aim of this study was to assess the beer wort fining efficiency of several hybrid carrageenans, carrageenans with different molecular weights and alkali treated carrageenans on different wort types. The polysaccharides used for testing were furcellaran, κ-carrageenan, κ/ι-hybrid carrageenan, ι-carrageenan and a hybrid carrageenan mix.

2. Materials and methods

2.1. Chemicals and polysaccharide preparations

All of the chemicals used were of analytical grade and purchased from Sigma-Aldrich unless stated otherwise. Furcellaran was extracted from the attached form of red algae *Furcellaria lumbricalis* collected from the coast of the Gulf of Finland (59°33'12.1"N, 24°50'35.0"E). The polysaccharide was extracted from the homogenized seaweed biomass in 20 mM sodium phosphate buffer (pH 9.0) at 95 °C for 3 hours followed by centrifugation and ethanol precipitation procedures described by Tuvikene, et al. (2010). Batches of κ-carrageenan, κ/ι-hybrid, ι- carrageenan and a hybrid carrageenan mix (made up of κ-, ι-, λ- and ν-carrageenans) were kindly provided by CP Kelco (Lille Skensved, Denmark). To remove low-molecular weight compounds (< 12 kDa) from the samples, carrageenan samples were dialyzed and converted to Na^+ form using ion exchange method described by Robal, et al. (2017).

The carrageenans were degraded to lower the molecular weight of the polymer in order to study the correlation between the molecular weight of the polysaccharide and the beer wort fining efficiency. The degradation was conducted using ultrasonic

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