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Development of novel quinoa-based yoghurt fermented with dextran producer *Weissella cibaria* MG1

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ABSTRACT

The aim of this study was to develop a novel beverage fermented with *Weissella cibaria* MG1 based on aqueous extracts of wholemeal quinoa flour.

The protein digestibility of quinoa based-milk was improved by applying complex proteolytic enzymes able to increase protein solubility by 54.58%. The growth and fermentation characteristics of *Weissella cibaria* MG1, including EPS production at the end of fermentation, were investigated. Fermented wholemeal quinoa milk using MG1 showed high viable cell counts ($> 10^9$ cfu/ml), a pH of 5.16, and significantly higher water holding capacity (WHC, 100%), viscosity (0.57 mPa s) and exopolysaccharide (EPS) amount (40 mg/l) than the chemical acidified control. High EPS (dextran) concentration in quinoa milk caused earlier aggregation because more EPS occupy more space, and the chenopodin were forced to interact with each other. Microstructure observation indicated that the network structures of EPS-protein improve the texture of fermented quinoa milk. Overall, *Weissella cibaria* MG1 showed satisfactory technology properties and great potential for further possible application in the development of high viscosity fermented quinoa milk.

1. Introduction

Consumer demand for cow's milk alternatives has increased as a result of people being intolerant to cow's milk, including lactose intolerance and cow's milk allergy. While lactose intolerance is triggered by the consumption of lactose-containing foods and a deficiency of lactase to enable lactose digestion, the cause cow's milk allergy is an abnormal immune response to one or more milk proteins (Crittenden and Bennett, 2005). Soy milk is the most common milk-substitute. However, 14% of the individuals who suffer from cow's milk allergy also have reactions to soy (Zeiger et al., 1999). Beside soy, other plants are used to develop non-dairy milk products, including oat, almond, coconut, rice and quinoa and their market is increasing. Even though the acceptance and consumption of these plant-based milk substitutes ("plant-based milks") is rising, many of these products have sensory characteristics that do not match the consumer preference in western countries (Jago, 2011; O.E. Mäkinen et al., 2015b; Monitor, 2005). Furthermore, plant-based milks, based on rice and other types of plants, have low or no protein content and are, therefore, not suitable to replace cow's milk or even soy milk (Jeske et al., 2017). Therefore, the development of new milk-substitute products that cause no adverse effects in humans and that have better nutritional, sensory and technological properties are necessary.

In general, the dairy substitute market is still growing: MarketsandMarkets (2017) estimated that the dairy alternatives market was valued at USD 7.37 Billion in 2016. It is projected to grow at a Compound Annual Growth Rate (CAGR) of 11.7% from 2017, to reach USD 14.36 Billion by 2022. One grain with such potential is quinoa (*Chenopodium quinoa*). It has been declared as "one of humanity's most promising crops" by the FAO, and it has been considered as a potential crop for NASA's Controlled Ecological Life Support System (Arendt and Zannini, 2013). Quinoa is an ancient crop that originates from the Andean region in South America (Jacobsen, 2003). It can grow from 1 m to 3 m tall and produces achenes as a fruit. These seeds are gluten-free, rich in starch and have a comparable chemical composition to cereal grains even though belong to the same botanic family of spinach and sugar beet. The protein content is remarkably higher than in true cereals, with literature values ranging from 12 to 23% (Abugoch James, 2009; Arendt and Zannini, 2013). Quinoa seeds also have significantly higher levels of minerals and some vitamins than true cereals (Arendt and Zannini, 2013; Jacobsen, 2003). The interesting nutritional quality of quinoa supports its inclusion in different food products. Many studies have however reported grassy and bitter off-flavours when used at high levels. Therefore, quinoa is usually used in only low concentrations

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(Ranhotra et al., 1993; Repo-Carrasco et al., 2003a).

Yoghurt is one of the most popular fermented milk products worldwide and has gained widespread consumer acceptance as a healthy food (Cruz et al., 2013). The impact of microbial exopolysaccharides (EPS) from lactic acid bacteria (LAB) on dairy products such as yoghurt, cheese, or milk based desserts has been extensively described in recent years (Mende et al., 2016). The improvement of the rheological properties by EPS is primarily due to the ability to bind water, reducing syneresis during storage, by increasing the viscosity of the serum phase, which leads to a higher taste perception (Lynch et al., 2018). In the dairy industry it is typically HePS-producing LAB that are used for fermentation, however, in this study, the HoPS-producing strain *Weissella cibaria* MG1 has been investigated for its impact on quinoa yoghurt-like production. Yet, little attention has been paid to the role of EPS in plant-based food and beverage industries. In recent studies, *W. cibaria* MG1 was used in barley malt wort fermentation (Zannini et al., 2013) and sourdough fermentation made from wheat or gluten-free flours (Wolter et al., 2014). In all these studies *W. cibaria* MG1 dextran formation ameliorate the structural and sensory characteristics of the investigated food matrices. The main objective of this research is to prototype a novel quinoa-based fermented yoghurt-like complementing bio-theological food processes able to deliver an innovative products mimicking, as much as possible, bovine milk yoghurt attributes. Prototyping such quinoa-based yoghurt-like product represents a key step to address the increasing demand for cow's milk alternatives, initially arising from people being intolerant to cow's milk, and, in recent years, due to particular lifestyles choices.

2. Materials and methods

Organic quinoa grains were obtained from Infinity Foods Co-operative Ltd. Chemicals were purchased from Sigma-Aldrich (St Louis, Missouri, USA) unless otherwise stated.

Commercial “Quinoa drink” was used as reference for the characterisation of the experimental wholemeal quinoa milk production. “Quinoa drink” is a commercial quinoa plant milk produced by EcoMil, with a labelled protein content of 1.5% and was purchased from a local store in Ireland.

Weissella cibaria MG1 was obtained from the culture collection of the Cereal Science laboratory of University College Cork, Ireland. *W. cibaria* MG1 has been previously identified as dextran hyper-producer (Galle et al., 2010) Working cultures from *W. cibaria* MG1 were prepared from glycerol stock stored at − 80 °C by streaking onto MRS agar (pH 5.8) (Meroth et al., 2003) containing 0.05 g/l bromocresol green (Dal Bello and Hertel, 2006). Plates were incubated at 30 °C for 48 h under anaerobic conditions. A single colony was inoculated into MRS broth and grown overnight at 30 °C prior to use.

2.1. Quinoa milk production and characterisation

The procedure for the quinoa-based milk production was adapted from the flowchart of rice-milk processing (Mitchell et al., 1988) summarised in Fig. 1.

Briefly, fifty grams of wholemeal quinoa flour, milled using a hammer mill (Mod. EM50, AMA Magico, San Martino in Rio, Italy) with a disc size of 1.5 mm, was mixed with 350 g water (ratio 1:7). The resulting suspension was autoclaved in glass bottles for 15 min at 121 °C to allow starch cooking and gelatinization. The suspension was blended in a semi-industrial blender (Titanium Major KMM020, Kenwood, Havant, United Kingdom) at medium speed for 6 min. Heat stable alpha-amylase Hitempase 2XP (Kerry Group, Ireland), at a concentration of 0.5% (w/w), was used for the dextrinization and liquefaction of starch. For starch degradation the suspension was heated to 50 °C for 20 min followed by 65 °C for 90 min. A conventional starch-iodine test was conducted to check the progress of starch breakdown. The suspension was left at 65 °C until the iodine test was negative followed by cooling to 25 °C and stirring for 40 min. The wholemeal quinoa flour suspension was mixed at medium speed for 6 min and bottled in previously sterilized 1 L glass bottles. High-pressure homogenisation treatments were carried out in a high pressure homogeniser (APV 1000, APV Homogenisers AS, Denmark) by applying 180 MPa.

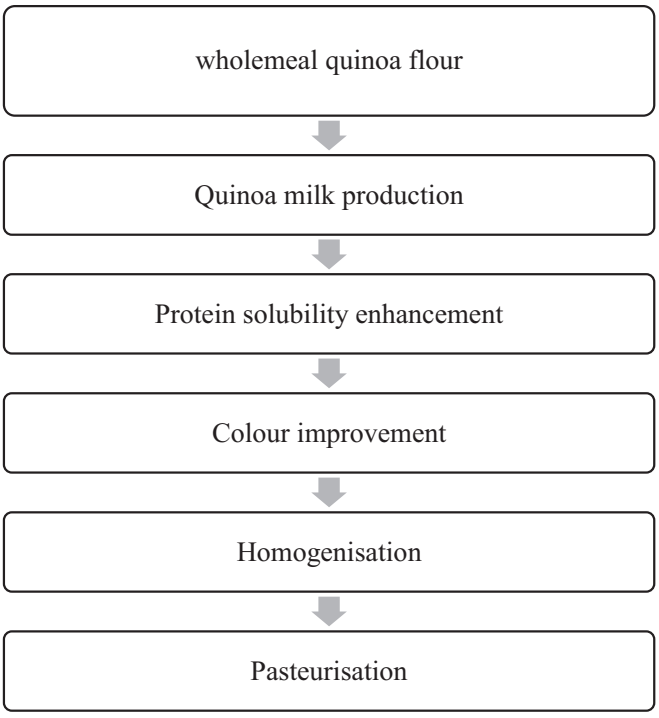

Fig. 1. Process steps for the quinoa-based plant milk production.

2.1.1. Protein content and protein solubility

The quinoa wholemeal flour and the resulting wholemeal quinoa milk were analysed according to the Kjeldahl method (MEBAK 1.5.2.1). The total nitrogen content was converted into protein content, using factor 6.25 (Matissek et al., 2014). In order to analyse the solubility of the proteins, samples were centrifuged at 3900*g* for 5 min. The protein contents of the supernatants were analysed using the Bensadoun and Weinstein (1976) modification of the Lowry assay (Lowry et al., 1951). The precipitation steps were not needed and therefore omitted. A calibration curve of bovine serum albumin was used to determine the concentrations. The results were expressed as % (w/w) of protein content in the supernatant of total protein content.

To enhance the protein solubility, the impact of different salt concentration, proteases and pH were evaluated.

2.1.2. Salt concentration

Salt solutions with NaCl concentration of 0, 0.025 and 0.05 mol/l was used in place of water for quinoa milk production using the procedure described above.

2.1.3. Protease treatment

The salt concentration with the highest protein solubility was chosen for the investigation of protease treatment. Two different proteases were tested to increase the protein solubility. Rustom et al. (1991) reported that papain increased the protein yield of peanut and soy milks. Purified papain preparation (Profix 100 L, Kerry Group, Ireland) and a complex proteolytic enzyme system, obtained from different microbial strains and plant species (Bioprotease PF50, Kerry Group, Ireland) were used. The recommended dosage (Profix 100 L: 4 g/Hl, Bioprotease PF50: 1 kg/tonnes) as well as a tenth and 10-fold of