

# The application of dextran compared to other hydrocolloids as a novel food ingredient to compensate for low protein in biscuit and wholemeal wheat flour

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**Abstract** Wheat is primarily used for bread-making. However, fungal diseases, grain moisture at harvest and low-protein contents strongly influence the quality of the wheat flour, thus creating challenges for traders, millers and commercial bakers who struggle to produce consistently high-quality products. This paper address the replacement of low-protein/wholemeal flour functionality for bread-making purposes. Three hydrocolloids, xanthan gum, dextran and hydroxypropyl methylcellulose, were incorporated into bread recipes based on high-protein flours, low-protein flours and coarse wholemeal flour. Hydrocolloid levels of 0–5 % (flour basis) were used in bread recipes to test the water absorption. The quality parameters of dough (farinograph, extensograph, rheofermentometre) and bread (specific volume, crumb structure and staling profile) were determined. Results showed that xanthan had negative impact on the dough and bread quality characteristics. HPMC and dextran generally improved dough and bread quality and showed dosage dependence. Volume of low-protein flour breads were significantly improved by incorporation of 0.5 % of the latter two hydrocolloids. However, dextran outperformed HPMC regarding initial bread hardness and staling shelf life regardless the flour applied in the formulation.

**Keywords** Xanthan gum · Dextran · HPMC · Bread quality

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## Introduction

Wheat is one of the major grains in the diet of a vast proportion of the world's population. Currently, the vast majority of global wheat produced is used for bread-making with most of the remaining finding use in pastas [1]. However, environmental conditions, crop management practices during grain development and post-harvest control strongly influence the quality of the wheat flour obtained [2]. This creates challenges for traders, millers and the commercial bakers who struggle to produce consistently high-quality products using the resulting wheat flour [3]. Particularly, northern Europe's generally temperate climate can have adverse effects on the crop. These negative attributes include diseases (especially those of fungal origin), grain moisture at harvest and low-protein contents, which collectively impede premium pricing of wheat grain for milling purposes (flour production) [4].

Additionally, other factors relating to the bread-making performance of flour also lead to variation in the bakers' purchase prices. One such parameter is water absorption, which is directly positively correlated with bread yield. Furthermore, both the colour and ash levels of the flours, which are both products of the bran content, also exert a pivotal role in the bread-making performance of the flour [5].

Wheat flour popularity as a raw material for staple baked goods arises from the properties of the final baked products. These attributes are gained through the formation of a viscoelastic dough during processing and gluten development, as well as gluten interactions with starch and other wheat flour components. Flour with high-protein contents, specifically gluten, is better suited to bread-making, resulting in a final baked product with a high-volume and fine open crumb structure [6]. The quality of the dough matrix

relies on sufficient gluten development (gas retention and oven-spring), due to the quality and quantity of gliadin and glutenin subunits, their ability to absorb water and interactions between dough constituents [7].

In the case of low-protein wheat flours, such as those often produced in northern European countries, it is necessary to increase the protein (gluten) content before incorporation into bread-making processes. To ensure standardisation of flour protein contents, it may be blended with high-protein raw materials resulting in a reliance on the availability of imported wheat and either domestic or external government policies, thus incurring price dependency issues [8]. Another approach involves flour fortification with vital wheat gluten, which may be obtained as a by-product of wheat fuel crop saccharification [5] or as a starch industry co-product [9]. However, these solutions are economically challenging to the milling industry and rely on a number of factors external to the control of the domestic industry. These include import constraints due to privatised sales and competition [10], environmental conditions [11], competition with non-food application of wheat crops [12] or delivery schedules.

As an alternative, weak (low-protein) wheat flour bread-making functionality can be increased using a range of processing aids such as oxidising agents like *L*-ascorbic acid or potassium bromate (its supplementation has been legally banned in several countries, while, in others, the industry has voluntarily decreased its use) [13, 14], enzymes such as tyrosinases, peroxidases, lipoxygenase, glucose oxidase and transglutaminase [15, 16], emulsifiers including diacetyl tartaric acid ester of monoglycerides [17] and hydrocolloids such as gums and fibres or their modified derivatives [18].

In the baking industry, various baking aids, preservatives and taste enhancers are added to bread-making formulations. This ensures that natural seasonal variations in the raw ingredients do not result unpredictability, which causes processing problems and may reduce end product consumer acceptance. The potential of hydrocolloids to address these problems is of growing importance. Their effects on dough matrices and in the baked bread can be associated with two main molecular functionalities: (1) the modification of water retention and (2) interactions with other dough constituents as gluten, non-gluten proteins, fibres and starch, which have a combined influence on the dough development or structural network during the bread-making process.

Several studies focusing on the potential use of hydrocolloids, such as hydroxypropyl methylcellulose (HPMC), xanthan gum,  $\kappa$ -carrageenan, in the bakery industries have been reported [19–22], and two comprehensive reviews have recently been published [23, 24].

The addition of hydrocolloids to wheat dough positively influences bread quality by increasing water absorption and thus dough rheology [25], retarding the staling due to a reduction in gluten–starch interactions [22] and increasing the viscoelastic properties of bread [26]. Hydrocolloids are also added to bakery products to increase the specific volume due to their ability to mimic the viscoelastic properties of gluten in bread dough [27]. In particular, for HPMC, it has been shown that, in wheat bread system, this hydrocolloid can improve the stability to the interface dough system during proofing by developing multiple interactions between the polymer and the bread constituents. These interactions confer additional strength to the gas cells throughout the baking process by reducing gas diffusion and losses, thereby conferring better volume and increasing moisture content of the final loaf [28–30].

Noteworthy, xanthan and HPMC, natural and chemically modified, respectively, are widely used hydrocolloids and serve as benchmark dough improvers alongside dextran, which is an EU-approved novel food ingredient whose potential in this role has not yet been fully characterised. Several studies have been performed to better understand the dextran structural properties [31], potential industrial applications [32], but only few studies evaluate the bread-making performance of dextran in wheat [33] and gluten-free systems [34].

As such, in this study, the functionality of both natural (xanthan and dextran) and modified (hydroxymethylpropylcellulose) hydrocolloids in various strength wheat flour (high-protein, low-protein and coarse wholemeal flours) recipes was investigated as a comparative study to address the problems associated with domestically available weak flours.

## Materials and methods

### Raw materials

Three different wheat flours, bakers' flour (high-protein content) (composition: carbohydrate 70.21/100 g, protein 12.36/100 g, fat 1.10/100 g, fibre 3.1/100 g, ash 0.87/100 g, moisture 13.23 %), biscuit flour (low-protein content) (composition: carbohydrate 73.68/100 g, protein 9.39/100 g, fat 0.85/100 g, fibre 3.0/100 g, ash 0.45/100 g, moisture 12.63 %) and wholemeal flour (Odlums, Portarlinton, Ireland) (composition: carbohydrate 58.89/100 g, protein 10.0/100 g, fat 7.40/100 g, fibre 9.0/100 g, ash 1.52/100 g, moisture 13.19 %), were used in this study. Protein, ash, fat and moisture contents were determined by AACC methods 46-10, 08-01, 30.25 and 44-15.02, respectively, for each flour. Vivapur<sup>®</sup> hydroxypropylmethylcellulose K4 M (HPMC) (J. Rettenmaier and Söhne, Rosenberg, Germany), KeltrolF xanthan gum (C.P. Kelco, USA) and

high molecular weight ( $5 \times 10^6$ – $4 \times 10^7$  Da) dextran (Bio-e.r.g., Jesi, Italy) were used as hydrocolloids in this study. Salt (Salt Union, Cheshire, UK) and dried yeast (Mauripan, Burns Philip Food Ltd., UK) were also incorporated into the bread-making recipes.

#### Hydrocolloid sample preparation and rheology

To analyse each hydrocolloid, 0.2 g was dissolved in 1 mL isopropanol at room temperature and was subsequently added to 20 mL water in the rheometer cup, using a concentric cylinder system (Anton Paar, Austria). The viscosity of each sample was measured over 10 min at 20 °C, using a constant shear rate of  $500 \text{ s}^{-1}$ .

#### Preparative dough analyses

Farinograph (constant flour method) and extensograph characteristics were determined according to the AACC Methods, 54-21 and 54-10, respectively. The following parameters were determined using a Brabender-farinograph (Duisberg, Germany): water absorption, percentage of water required to yield dough consistency of 500 Brabender units (BU), dough development time (DDT, time to reach maximum consistency), stability (length of time for which dough consistency is at 500 BU), mixing tolerance index (MTI, consistency difference between maximum peak height and peak height recorded 5 min later) and elasticity (bandwidth of the curve at the maximum consistency). After 50 mm stretching, the Brabender extensograph was used to measure the resistance to constant deformation ( $R_{50}$ ), extensibility ( $E$ ) and  $R_{50}/E$  ratio.

The Chopin rheofermentometre (Villeneuve-Garenne, France) was used to measure dough development. Displacement of a 1,500 g weight by the rising dough (300 g) was measured over 3 h at 30 °C and was directly related to the volume of gas produced, thereby allowing calculation of the dough gas retention capabilities. Dough was prepared as for the baking studies (reported below).

#### Bread-making and analyses

On a 100 % flour basis, the dough recipe contained water (calculated from farinograph, reported in Fig. 2), and 2.0 g each of salt and yeast, 1.5 g sugar, 3.0 g fat, 0.05 g ascorbic acid, and 0.1 g sodium stearyl lactylate supplemented with 0 (control), 0.5, 1.0, 2.5 or 5.0 % of dextran, xanthan gum or HPMC. The dough was prepared by weighing out the dry ingredients, excluding yeast which was incorporated into the water at 30 °C and subsequently added to the other ingredients. Everything was combined in a mixer (Kenwood Chef Classic KM336) at speed I for 60 s followed by scraping down the sides of the bowl and at speed II for 90 s.

After mixing, the dough was rested in a proofer (Koma, Koeltechnische, The Netherlands) at 30 °C and 85 % relative humidity for 15 min before it was divided into 450 g portions and placed in non-stick baking tins (180 mm × 120 mm × 60 mm, 454 g tins, Sasa UK, Mid-dex, UK). The dough was then proofed for 60 min under the same conditions and baked immediately in a preheated deck oven (MIWE condo oven, MIWE Michael Wenz GmbH, Arnstein, Germany) at 220 °C top and bottom heat for 22 min. The oven was steamed (1,000 mL) before loading and again on loading the bread. For staling experiments, loaves were packed into plastic bags 120 min after baking, when adequately cooled, which were sealed and subsequently stored at room temperature ( $25 \pm 2$  °C) for 2 and 5 days. Baking was performed on three different days (three independent trials), and nine loaves were prepared for each bread type at each baking trial.

#### Bread analyses

After cooling, volume of the loaves was measured using a VolScan Profiler (Stable Micro Systems, Surrey, UK), and specific volume was calculated by dividing the loaf volume by its mass. Additionally, bake loss was determined (subtracting loaf weight from pre-baked dough weight) and calculated as a percentage using the VolScan software. Texture analysis was conducted according to AACC method 74-09 [35] and performed on day 1 (120 min after baking), 2 and 5 days after baking using texture profile analysis (TPA) tests with a TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 25-kg load cell and a 20-mm aluminium cylindrical probe. The settings used were a test speed of  $2 \text{ mm s}^{-1}$  with a trigger force of 20 g to compress the middle of the breadcrumb to 40 % of its original height. For TPA analysis, four slices of 25 mm thickness were sliced from the centre of each of three loaves of each bread type, from three separate batches. The staling rate was calculated as increase in hardness after 5 days of storage [staling rate = (hardness (day 5 – day 0)/days of storage)]. Results were analysed using Texture Expert 1.17 software (Stable Micro Systems), and values for hardness, springiness, and chewiness were calculated using the associated software.

Crumb structure was evaluated by image analysis using C-cell Imaging System and associated software (Calibre Control International Ltd., UK). The parameters used were cells/cm<sup>2</sup> (number of cells/slice area), wall thickness and net cell elongation (degree of overall elongation).

#### Statistics

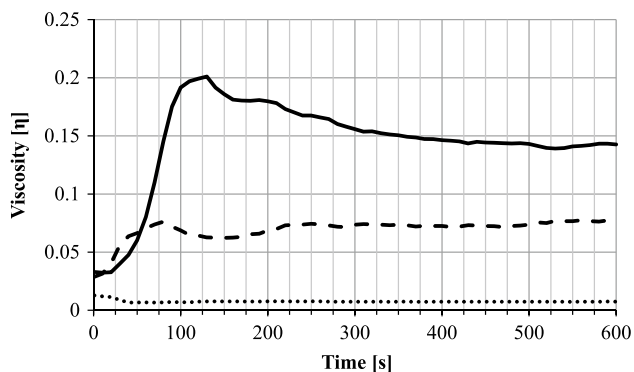
All determinations were performed in triplicate, and the average result is presented. The standard baking tests were

performed on three loaves for each bread type at each of three separate baking trials. The Excel Analysis Tool-Pak (Microsoft Corporation®) was used for statistical calculations.

## Results and discussion

### Hydrocolloid viscosity

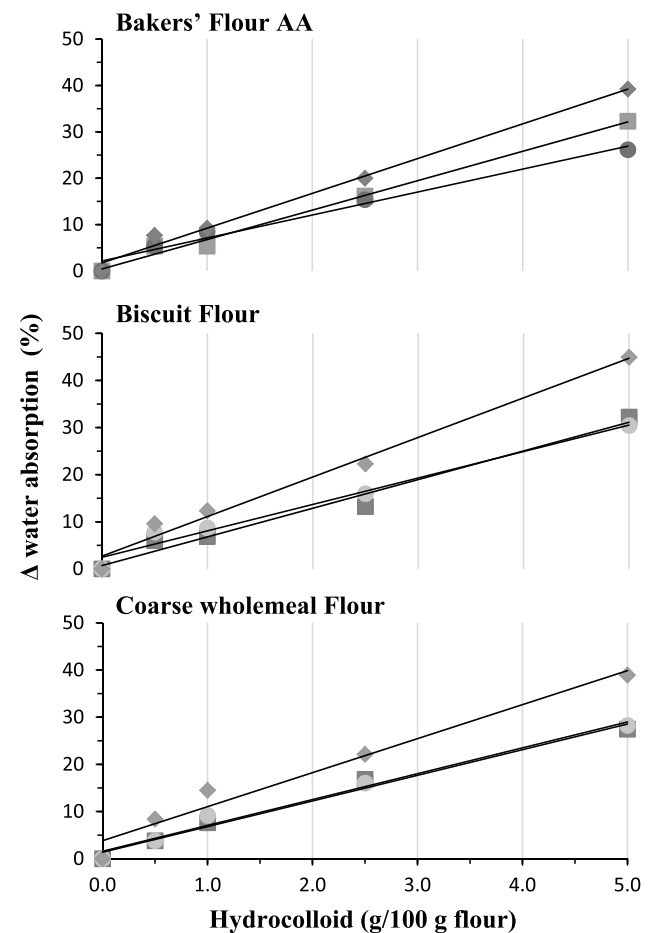
Amongst the three hydrocolloids studied, xanthan has the highest viscosity followed by HPMC and dextran (Fig. 1). The latter shows a constant viscosity profile over the 10 min of analyses under constant shear rate ( $500 \text{ s}^{-1}$ ). After hydration, a similar behaviour was observed for dextran but at a lower overall viscosity than HPMC. Additionally, the hydration rate of dextran was accelerated when compared to HPMC and particularly xanthan (Fig. 1). Structurally, dextran is an  $\alpha$ -1,6-linked glucose-based polysaccharide with various combinations of  $\alpha$ -1,2,  $\alpha$ -1,3 and/or  $\alpha$ -1,4 branching leading to a globular formation, which is dense and can exist as a spherical suspension in a complex matrix, such as dough [36]. These properties aid macromolecule mobility and low viscosity (Fig. 1) mimicking Newtonian behaviour [37]. However, after the initial hydration of xanthan (110 s), the slope of the viscosity curve decreased slowly over time until a plateau was reached after approximately 440 s of measurement. The unfolding of the xanthan macromolecular structure under shear stress resulted in its high viscosity, thus potentially limiting the mobility of the solution and leading to a low-density, high-volume pseudoplastic-type behaviour [38]. The viscosity of HPMC was intermediate between xanthan and dextran, likely due to its water-binding properties discussed below (“Hydrocolloid performance in dough system” section).



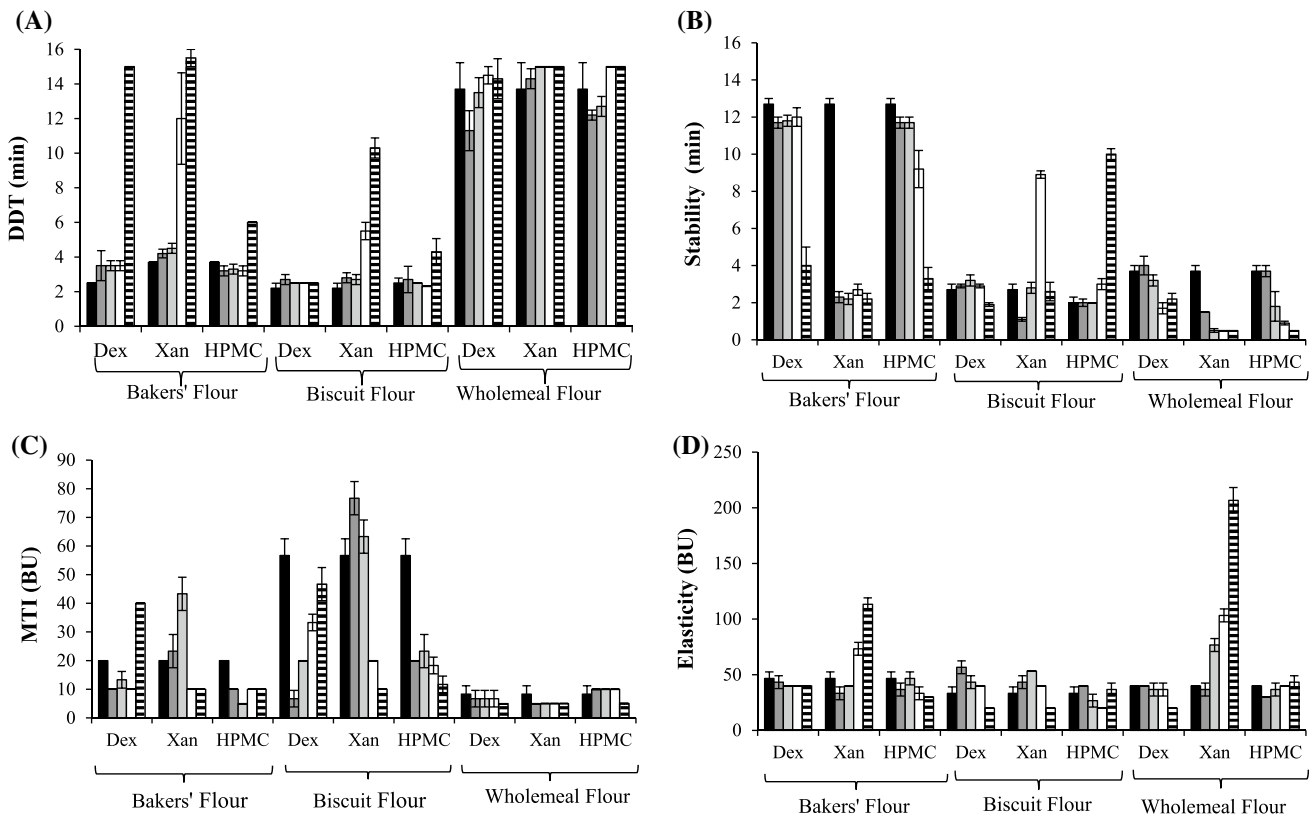
**Fig. 1** Viscosities over time under constant shear rate ( $500 \text{ s}^{-1}$ ) for dextran (dotted line), xanthan (solid line) and HPMC (dashed line)

### Hydrocolloid performance in dough system

Water absorption was positively correlated with hydrocolloid addition in a linear manner ( $R^2 \geq 0.97$ ), regardless of whether HPMC, xanthan or dextran, were used, in each of the three flours analysed by the farinograph (Fig. 2). Of the hydrocolloids tested, HPMC absorbed the greatest volume of water followed by dextran and xanthan, respectively, though the latter two were indistinguishable in biscuit and wholemeal flour analyses. For HPMC, the water-binding capacity was greater due to this hydrocolloids' polar hydroxyl group structure, which promoted increased water interactions through hydrogen bonding [39, 40]. Irrespective of the flour type, increasing levels of hydrocolloid resulted in a higher dough development time (DDT), due to the increased time needed for gluten hydration, except for dextran in biscuit flour whose DDT was unaffected. In biscuit flour, due to the low-protein content, dough development is dependent on the hydrocolloid used



**Fig. 2** Change in water absorption of formulation dependant on flour/hydrocolloid ratio, as measured by the farinograph. Hydrocolloid used included dextran (solid rectangle), xanthan (solid circle) and HPMC (solid diamond). The  $R^2$  value for all plots  $\geq 0.97$



**Fig. 3** Farinograph analyses of bakers', biscuit and wholemeal flours with dextran (dex), xanthan (xan) and HPMC illustrating **a** dough development time (DDT), **b** dough stability, **c** mixing tolerance index (MTI) and **d** dough elasticity for the control (black bar), 0.5 % hydro-

ocolloid dosage (dark grey bar), 1.0 % hydrocolloid dosage (light grey bar), 2.5 % hydrocolloid dosage (white bar) and 5.0 % hydrocolloid dosage (striped bar)

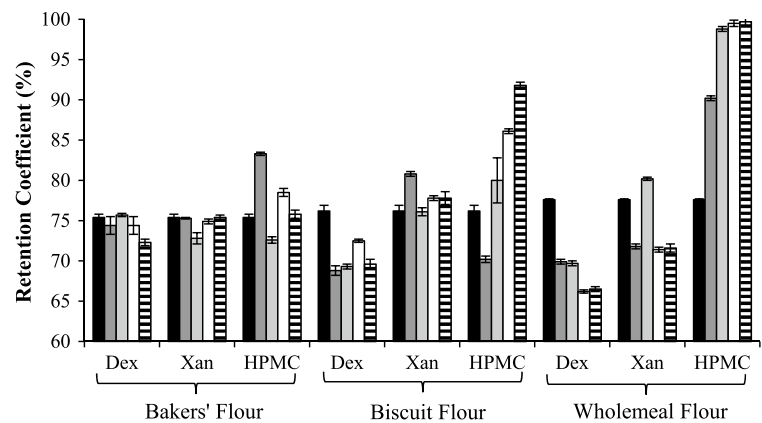
rather than solely on the gluten development. Additionally, since dextran displayed relatively rapid hydration (Fig. 1), the lag period is eliminated and the time necessary to reach a dough consistency of 500 BU is significantly reduced. Even though dough development is heavily dependent on the type of hydrocolloid used, at lower dosages (0–1.0 %) there is minimal difference between the hydrocolloids and their relationships with dough development time (Fig. 3a). Furthermore, dough stability is negatively correlated with hydrocolloid addition with the exception of the biscuit flour where increasing the dosage generally tended to improve stability, the only exception was dextran, which had little dose-dependent effect (Fig. 3b). A strong gluten network ensures dough stability, and in general, it is likely that the disruption of this matrix in the high-protein flours (bakers' and wholemeal flours) by increased hydrocolloid addition is responsible for the negative dose–response correlation. However, in the low-protein biscuit flour, the electrostatic interactions of both xanthan and HPMC with the proteins support the weak gluten network [41, 42], thus explaining the observed positive correlation between dough stability and

hydrocolloid dose, up to a certain hydrocolloid-specific level of saturation (Fig. 3b).

The mixing tolerance index (MTI) of the wholemeal dough was unaffected by addition of any of the hydrocolloids at all dosage levels tested due to the disruptive interaction of fibre with the gluten network [43]. However, bakers' flour MTI was generally negatively correlated with hydrocolloid addition, with dextran proving the exception at lower addition levels (Fig. 3c). Furthermore, biscuit flour dough MTI was negatively correlated with the addition of xanthan (at low doses), but conversely was positively correlated with HPMC addition and dextran addition at lower dosages (Fig. 3c). The increased stability of the dough (lower MTI value) can be attributed to the strengthening effect of hydrocolloid addition [39], in agreement with the dough stability data (Fig. 3b). Dough elasticity measurements revealed that xanthan addition had a negative effect for bakers' and wholemeal flour dough, but had the opposite impact on biscuit flour at most levels of addition. This is regarded as a negative attribute as the dough viscoelastic ratio is altered when compared to the control samples. Dextran and HPMC generally do not have a strong influence on



**Fig. 4** Rheofermentometre analyses of bakers', biscuit and wholemeal flour dough with dextran (dex), xanthan (xan) and HPMC illustrating gas retention coefficient for the control (black bar), 0.5 % hydrocolloid dosage (dark grey bar), 1.0 % hydrocolloid dosage (light grey bar), 2.5 % hydrocolloid dosage (white bar) and 5.0 % hydrocolloid dosage (striped bar)



this parameter relative to xanthan (Fig. 3d), thus maintaining viscoelastic dough with properties, which are similar to the control dough.

The  $R_{50}/E$  ratio, directly related to the flour functionality (gluten strength and dough extensibility), was generally not affected when dextran and HPMC were included in bakers' and biscuit flours, independent of the concentration used (data not shown). This low  $R_{50}/E$  ratio is desirable in a dough system as it promotes good oven-spring resulting from strong gas retention properties. This strong gas retention was also observed in HPMC performance in rheofermentometre trials (Fig. 4) and in previous studies [39]. Conversely, in wholemeal dough, both dextran and HPMC slightly and significantly increase the  $R_{50}/E$  ratio (data not shown), respectively, mainly due to a reduction in elasticity. Regardless the type of flour investigated, higher additions of xanthan resulted in a significant  $R_{50}/E$  ratio increase due combined increased  $R_{50}$  and decreased  $E$ .

#### Gas retention capabilities (dough development)

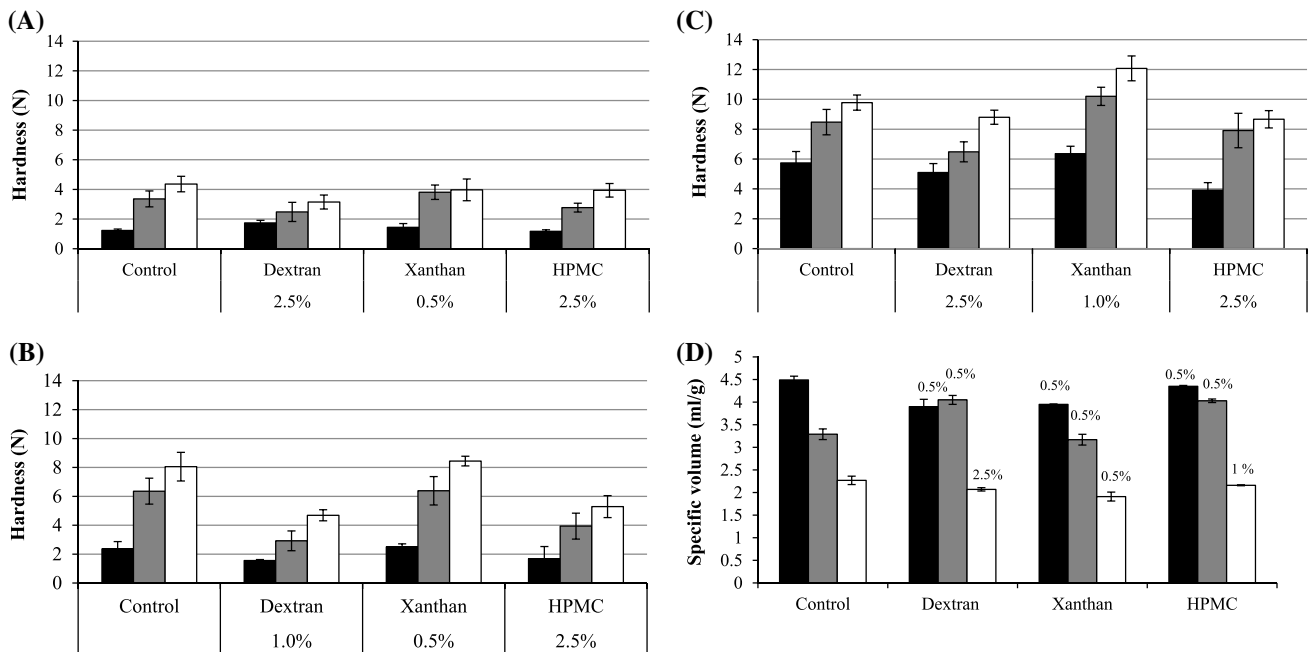
Regarding the gas behaviour, the retention coefficient describes the dough ability to be stretched into thin membranes and, in turn, is associated with protein network quality. Gas retention during fermentation is indicative of the dough development potential and the tested flours all behaved similarly (Fig. 4). In bakers' flour, there is no significant impact coming from the incorporation of dextran or xanthan, generally. However, generally when HPMC is used in this system, the retention coefficient of the dough increases inversely from the lowest level of incorporation (excluding the 1 % HPMC formulation) (Fig. 4). In the biscuit flour system, dextran incorporation decreases the dough retention coefficient, while xanthan has no statistical impact on this parameter with the exception of 0.5 % incorporation, which results in improved dough development. HPMC inclusion in the biscuit and wholemeal flour formulations was positively correlated with the retention coefficient and thus improved dough development. However,

in wholemeal flour dough, both dextran and xanthan had a negative affect at most dosage levels. Overall, HPMC showed the biggest improvement on the quality of wholemeal flour dough than any of the other hydrocolloid flour combination.

These results support the use of HPMC as an improver of gas retention during fermentation. As previously reported by Guarda, Rosell, Benedito, & Galotto [19], a possible explanation is that HPMC gives more stability at the gas–dough interface during proofing and confers additional strength to the gas cells during baking, thus increasing the gas retention leading to greater volume. HPMC surface activity tends to stabilise the dough foam through dispersal of bubbles. This results in an even distribution of smaller gas cells, which, in addition to increasing viscosity, confer structural stability particularly at the gas–liquid interface where an elastic micro-gel is formed [44]. Conversely, the dough volume was decreased when microbial hydrocolloids, xanthan and dextran, with relatively lower water absorption properties (than HPMC) were used. The authors hypothesise that in the case of xanthan, its anionic properties likely interfere with the positively charged proteins, thus impeding the role of the otherwise elastic gas-retaining gluten film. Conversely, the large polymeric distribution of dextran may contribute a physically disruptive barrier to the gas–liquid interface and overall dough system, thus limiting free expansion during fermentation.

#### Bread analyses

Irrespective of the flour used, specific volumes of the breads were generally negatively correlated with, or did not affect, bread volume when any hydrocolloid was incorporated into the formulations. However, using 0.5 % dextran or HPMC resulted in significantly increased specific loaf volumes for biscuit flour formulations (Fig. 5d). These results suggest that xanthan, due to its strong gluten–hydrocolloid interactions, may limit dough extension. However, in the case of HPMC, this strengthening property leads to stabilisation of



**Fig. 5** Hardness, staling and specific volume of wheat breads. The initial hardness (*black bar*) and staling values on days 2 (*grey bar*) and 5 (*white bar*) of breads made using **a** bakers' flour, **b** biscuit flour and **c** wholemeal flour, incorporating the optimal hydrocolloid level (shown *below bars*) and the control breads with no hydrocolloids

added. **d** The specific volumes of breads made using bakers' (*black bar*), biscuit (*grey bar*) or wholemeal flours (*white bar*), incorporating the optimal hydrocolloid level (shown above *bars*) and the control breads with no hydrocolloids

the gas cell without compromising elasticity and expansion potential, thus resulting in a higher loaf volume [20]. Conversely, at higher addition levels, dextran does not retain as much gas as HPMC, but it also does not limit dough expansion due to a lack of electrostatic or ionic interaction with the gluten network, thereby allowing higher final bread volume. Dextran has previously been hypothesised to increase loaf volume by increasing the water-binding capacity of the dough and influence formation of the gluten through H-bonding or steric interactions [45]. At optimal dosage levels (0.5 %) of HPMC or dextran, the hydrocolloid performances were very similar or indistinguishable over most parameters analysed (DDT, dough stability, MTI, elasticity,  $R_{50}/E$  and specific volume).

From a textural perspective (Fig. 5a–c), there is a trend towards a reduction in initial loaf hardness when dextran (1.0 %) and HPMC (2.5 %) are incorporated into the formulation, but the differences are not statistically relevant. However, the staling profile of the loaves over 2- and 5-day storage periods shows that the incorporation of dextran or HPMC results in a softer loaf than the control. The textural amelioration of breads due to HPMC incorporation has been previously published [39] and is likely due to the high fermentation gas retention (Fig. 4), better water absorption (Fig. 2) and redistribution of dough matrix components

(gas cells and starch) before baking commences [19, 44]. The successful application of dextran as a bread texture improver has been reported using an in situ sourdough application [46]. However, its functionality as a bread-making ingredient has been less frequently explored, due to its novel food ingredient status [47]. Conversely, xanthan performs poorly from initial loaf hardness and staling viewpoints, likely due to a cell wall thickening effect [39].

Using any of the three flours investigated in this study, crumb structure was generally unaffected by hydrocolloid addition when considering number and area of cells. However, when using bakers' flour formulations, the wall thickness of the crumb was significantly decreased upon addition of any hydrocolloid to the same extent regardless of the hydrocolloid used or its dosage (0.5–5.0 %) (results not shown). When biscuit flour formulations were considered, cell wall thickness was also significantly reduced by HPMC addition to the same extent regardless of dosage (1.0–5.0 %) (results not shown). For wholemeal flour dough, incorporation of any hydrocolloid at a dosage level of 0.5 or 1.0 % resulted in a significantly thinner cell wall. In general, dextran displayed a trend towards a positive dose–response with wall thicknesses like HPMC; however, xanthan functioned in the opposite manner (results not shown).

## Conclusion

In general, xanthan always performed relatively poorly in all wheat flour formulations, which may be related to its higher viscosity or its slower water absorption rate than either dextran or HPMC. As such, the incorporation of dextran or HPMC at 0.5 % dosage levels is a viable option to increase the specific volume in refined (bakers' and biscuit) wheat flours. However, dextran outperformed HPMC regarding bread initial hardness and staling shelf life at the lowest dosage level for biscuit flour or at 2.5 % for both hydrocolloids using bakers' or wholemeal flours. Additionally, the incorporation of clean label dextran as a novel food ingredient is the preferred hydrocolloid option.

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**Conflict of interest** None.

**Compliance with Ethics Requirements** This article does not contain any studies with human or animal subjects.

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