



Substitution of potassium sorbate preservative by fermented wheat flour in the production of biscuits. Safety, nutritional and sensory characteristic potentials

R. Fink¹  and S. Filip^{2,3*} 

¹ Faculty of Health Sciences, University of Ljubljana, Zdravstvena pot 5, 1000 Ljubljana, Slovenia

² Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

³ HQF Solutions d.o.o., Podutiška cesta 76, 1000 Ljubljana, Slovenia

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ABSTRACT

In this study, the potential of the substitution of the conventional preservative potassium sorbate by fermented wheat flour as an alternative was analysed. The organic acid content and antibacterial activity of preservatives and the macronutrient characterisation were tested, as was the sensory evaluation of biscuits with added conventional and alternative preservatives. The results show that the fermented wheat flour contains secondary fermentation metabolites (e.g. acetic, lactic and pyruvic acids). Both fermented wheat flour and potassium sorbate have antibacterial effects against *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis*. The sensory evaluation demonstrated that fermented wheat flour in the recommended concentration range had no impact on sensory quality. Therefore, fermented wheat flour represents a potential substitution for conventional potassium in bakery products.

KEYWORDS

fermented wheat flour, potassium sorbate, preservatives, antibacterial, biscuits, sensory evaluation

* Corresponding author. E-mail: sebastjan.filip@hqf-solutions.eu

1. INTRODUCTION

Production technologies, consumers' perceptions and increased awareness of emerging pathogens have forced the food sector to assess the quality and safety of their products and respond accordingly (Marriott et al., 2018). Bacterial contaminants have been considered a significant hazard for both food safety and food spoilage worldwide. Moreover, microbial spoilage causes the deterioration of approximately a quarter of the world's food supply (FAO, 2011). Good manufacturing practice recommends using chemical compounds to prevent food spoilage and foodborne illnesses (Marriott et al., 2018). For example, potassium sorbate (PS) has been used as an effective food preservative in several food sectors, especially for bakery products. The anti-bacterial activity of PS is related to the carboxyl group $-\text{COOH}$ and the number of carbon atoms (Türe et al., 2012). Wang et al. (2018) tested different food preservatives and found PS to be efficient against *Escherichia coli* and *Staphylococcus aureus*. Even though PS did not show any significant public health issues, studies demonstrated that PS should be used with precaution (Mamur et al., 2010; Carochio et al., 2014). Accordingly, this study aimed to analyse the potential of fermented wheat flour (FWF) as a preservative for bakery products concerning safety, sensory aspect and food composition.

2. MATERIALS AND METHODS

2.1. Fermented wheat flour and potassium sorbate

A sample of FWF was supplied by Lesaffre (France). According to the supplier's specification, FWF contains 3% proteins, 36% carbohydrates and <5% fats. The nutritional value per 100 g is 214 Kcal. For application, FWF has to be directly mixed with flour in concentrations from 0.7% to 1.75%. PS (E 2022) was provided by Brentag (Slovenia).

2.2. Quantification of organic acid compounds by HPLC analysis

In the present research, 1 g of FWF was diluted in 50 mL of distilled water and mixed with a magnetic stirrer at 200 r.p.m. for 15 min. Standard solutions of pyruvic, acetic and lactic acids were prepared in-stock solutions as 1 g L^{-1} and diluted in the concentration range from 50 mg L^{-1} to 1 mg L^{-1} . The acids were determined by HPLC (Agilent Technologies 1100 Series) unit with an LC-10 AT-VP pump, UV-VIS photodiode array detector (DAD) and Ascentis express C18 HPLC, $5 \mu\text{m} \times 4.6 \text{ mm} \times 150 \text{ mm}$ column. The mobile phase consisted of $\text{H}_2\text{SO}_4:\text{H}_2\text{O} = 0.2:99.8$ and the flow rate was adjusted to 1 mL min^{-1} . The analytical runs were performed at 45°C , and the detector was set to 210 nm.

2.3. Antimicrobial potential of fermented wheat flour and potassium sorbate

The antimicrobial potential of FWF and PS was analysed by assessing the minimal inhibitory concentration (MIC). In this experiment, the microdilution method was used as described by the Clinical and Laboratory Standard Institute (CLSI, 2018). Standard strains of *E. coli* ATCC 35218, *S. aureus* ATCC 25923, *Bacillus cereus* CCM 2010 and *Bacillus subtilis* WDCM 00003 were used. Bacterial cells were harvested in the logarithmic phase of growth and diluted in nutrient broth to the concentration of $1 \times 10^6 \text{ CFU mL}^{-1}$. The stock of PS was added to 10 mL of nutrient broth



to achieve a final concentration of 20 mg mL⁻¹. In the next step, 100 µL of two-fold serial dilutions of PS was added to sterile 96-well microplate to achieve final concentrations of PS ranging from 0.37 to 20 mg mL⁻¹. Equal amounts of bacterial suspension containing 0.9% NaCl were used as the negative control. FWF was diluted in the same way to final concentrations ranging from 0.13 to 70 mg mL⁻¹. After incubation at 37 °C for 24 h, the lowest concentration with no visible growth of tested bacteria was defined as MIC (Wang et al., 2018). All experiments were carried out in three parallel and three repetitions.

2.4. Preparation of biscuits with added fermented wheat flour and potassium sorbate

For the sensory evaluation, the most basic recipe for biscuits was used, containing only three main components: wheat flour, fat (margarine) and sugar in a ratio of 3:2:1 with the addition of different concentrations of FWF and PS. A standard recipe without preservative represented our reference. Additionally, 0.2% of PS and 0.65, 1.25, 2.5 and 5.0% of FWF were added. The proportion of ingredients was maintained in all formulations. After the dough was mixed in a spiral kneader, it was rolled with a rolling pin to a thickness of 5 mm and cut in round biscuits using a cylinder (diameter: 2.5 cm) then baked at 165 °C for 12 min.

2.5. Macronutrient determination and physical and chemical characteristics of the biscuits with added fermented wheat flour and potassium sorbate

Moisture, fat, protein, ash and total carbohydrate contents were determined in triplicate using standard methods reported by the Association of Official Analytical Chemists (AOAC) (AOAC, 2000). The protein content was calculated from nitrogen using the factor of 6.25 as reported by Islas-Rubio et al. (2014). Carbohydrate and energy values were calculated according to the AOAC (2000). Total titratable acidity (TTA) was determined by homogenising 10 g of sample with 90 mL of distilled water and express as the amount (mL) of 0.1 M NaOH to obtain a pH of 7. The pH values were measured with a Foodtrode electrode (Hamilton, Switzerland).

2.6. Sensory evaluation of biscuits with added fermented wheat flour and potassium sorbate

2.6.1. Quantitative descriptive analysis. The quantitative descriptive analysis (QDA) sequential monadic method (Stone et al., 2012) was carried out by a semi-trained panel of eight members (26–39 years old, gender distribution 50/50) with knowledge and experience in general descriptive analysis and previous experience in the evaluation of biscuits. In order to obtain a complete description of a product's sensory profile, the assessors used a discrete intensity scale from 1 to 9 for selected sensory descriptors: odour, taste, flavour and texture perceived in the mouth (Pasqualone et al., 2019) (Table 1).

2.6.2. Hedonic test. A total of 26 subjects, regular biscuits consumers (8 male and 18 female), with general knowledge in sensory science and limited practical experiences of formal sensory evaluation took part in the study. Thirteen parameters within four sensory categories: odour, taste, flavour and texture, which define the product's quality, were assessed. A nine-point hedonic scale (1 low acceptance; 9 high acceptance) was used, and panellists were instructed to compare the samples to the control.



Table 1. Descriptive terms used for sensory evaluation of biscuits

Descriptor	Definition	Scale anchors	
		Min (0)	Max (9)
Odour	Smell of baked biscuits with the typical smell of sweet, slightly caramel and baked flour, bread crumb	Odourless, unpleasant odour of sour, fermented, burned	Intensive smell of baked biscuits with the slightly sweet and pleasant odour
Taste	Slightly sweet taste of biscuits, taste of margarine sunflower fat, pleasant taste of baked flour and breadcrumbs	Tasteless, unpleasant taste, sour, fermented, like spoiled acid bread	Pleasant taste of baked flour with sweet taint and pleasant taste of margarine sunflower fat
Flavour	Typical flavour of homemade biscuits baked with wheat flour	Unpleasant flavour of fermented, sour, wet wheat flour	Intensive flavour of baked wheat flour with sunflower fat
Texture	Slightly dry fatty surface, crumbliness between fingers and in the mouth while chewing with graininess	Wet, plastic between fingers, no crumbliness, no graininess, intensive mouth coating	Crumbliness typical for biscuits, no mouth coating, very grainy, leaving differently sized crumbs

2.7. Statistical analysis

Statistical analysis was run on R software, version 3.6.3 (Bell Laboratories, New Jersey, U.S.). Data normality was checked using the Shapiro–Wilk test ($P > 0.05$). As a multiple comparison test, we used Duncan test. To mitigate the issue of an inflated type I error rate because of multiple comparisons, we first ran a one-way ANOVA. If ANOVA was statistically significant ($P < 0.05$), we ran a Duncan test to confirm the pairwise differences at a significance level of 5%; otherwise, we did not reject the null hypothesis of no difference across the means.

3. RESULTS AND DISCUSSION

3.1. Quantification of organic acid compounds of fermented wheat flour

The results show that among acids acetic acid is the most predominant (2.22 g/100 g), followed by lactic acid (1.67 g/100 g) and pyruvic acid (0.04 g/100 g). Likewise, [Mohsen et al. \(2016\)](#) demonstrated that lactic acid, acetic acid and pyruvic acid are secondary metabolites of wheat fermentation. Our findings are also consistent with those of [Al-Jassaci et al. \(2016\)](#), who reported acetic acid to be a predominant secondary metabolite of wheat fermentation.

3.2. Antibacterial potential of fermented wheat flour and potassium sorbate

The lowest PS concentration for bacteria inhibition was found in the case of *S. aureus* (2.5 mg mL⁻¹), followed by *B. cereus* and *E. coli* (5 mg mL⁻¹) and *B. subtilis* (10 mg mL⁻¹). In the case of FWF, the lowest MIC was found for *S. aureus* (2.2 mg mL⁻¹), *B. cereus* (8.8 mg mL⁻¹) and *B. subtilis* (17.5 mg mL⁻¹); however, the impact on *E. coli* was not determined ([Table 2](#)).



Table 2. Antibacterial activity of PS and FWF expressed as MIC (mg mL⁻¹) by microdilution methods for selected bacteria

Bacterium	PS MIC (mg mL ⁻¹)	FWF MIC (mg mL ⁻¹)
<i>E. coli</i> ATCC 35218	5.00	ND
<i>S. aureus</i> ATCC 25923	2.50	2.18
<i>B. cereus</i> CCM 2010	5.00	8.75
<i>B. subtilis</i> WDCM 00003	10.00	17.50

Legend: MIC: minimal inhibitory concentration; ND: not determined; FWF: fermented wheat flour; PS: potassium sorbate.

The results coincide with the findings reported by Raftari et al. (2009), who tested acetic acid, lactic acid, propionic acid and formic acid against *S. aureus* and *E. coli* and found that *S. aureus* was more susceptible to acids than *E. coli*. Also, Katina et al. (2002) studied lactic acid in wheat sourdough bread and found that a concentration of 1.7% was effective against *B. cereus* spores.

3.3. Macronutrient determination and physical and chemical characteristics of biscuits with added fermented wheat flour and potassium sorbate

The results (Table 3) show that adding FWF or PS to biscuits does not affect protein and fats ($P > 0.05$) contents, but demonstrated a statistically significant impact on ash, water, carbohydrates, TTA and pH ($P < 0.05$). Increasing the FWF concentration increases ash content in samples from 0.78 to 2.71 g/100 g on average ($P < 0.05$). Similar to that, we found that adding FWF in biscuits increased TTA from 3.66 to 4.40 on average ($P < 0.05$) and decreased pH from 6.78 to 5.47 ($P < 0.05$) (Table 3). A change in TTA and pH corresponds to organic acid compounds and consequently to antibacterial assessment. These findings also confirm those of Katina et al. (2002), who found wheat sourdough bread to have low TTA, low pH and longer shelf life due to secondary fermentation metabolites.

3.4. Sensory evaluation of the biscuits with added fermented wheat flour and potassium sorbate

3.4.1. Quantitative descriptive analysis. The results (Table 4) show that the addition of FWF significantly affects the odour, taste, flavour and texture; nevertheless, panel members did not detect significant difference between reference and sample with 0.65% FWF (Table 4) in any descriptors. The addition of 0.2% PS had a significant effect on odour, taste and flavour. The addition of 0.65% and 1.25% of FWF has no significant effect on odour and flavour; therefore, we are quite confident that it would be difficult for consumers to detect differences compared to the reference sample. The addition of 1.25% FWF has a statistically significant effect on taste, which may influence consumers.

3.4.2. Hedonic test. The addition of FWF has a significant influence on different sensory characteristic of the investigated samples (Table 5). The addition of FWF or PS has no significant influence on overall intensity and margarine odour, the overall intensity of flavour,



Table 3. Physical and chemical characteristics of the biscuits with added FWF and PS

Sample	Proteins (g/100 g)	Fats (g/100 g)	Ash (g/100 g)	Water (g/100 g)	Salt (g/100 g)	Carbohydrates (g/100 g)	TTA	pH
Reference	7.45 ± 0.05 ^a	26.40 ± 1.01 ^b	0.47 ± 0.01 ^f	1.96 ± 0.01 ^d	ND	63.72 ± 0.09 ^a	3.33 ± 0.11 ^d	7.21 ^a
PS 0.2%	7.24 ± 0.06 ^a	27.82 ± 0.99 ^a	0.58 ± 0.02 ^e	2.65 ± 0.03 ^a	ND	61.71 ± 1.00 ^b	3.60 ± 0.20 ^c	7.06 ^b
FWF 0.63%	7.41 ± 0.03 ^a	27.82 ± 0.40 ^a	0.78 ± 0.01 ^d	2.13 ± 0.02 ^c	ND	61.85 ± 0.86 ^b	3.66 ± 0.11 ^c	6.78 ^c
FWF 1.25%	7.75 ± 0.02 ^a	27.35 ± 0.80 ^{ab}	1.04 ± 0.01 ^c	2.20 ± 0.01 ^b	ND	61.66 ± 0.64 ^b	3.93 ± 0.11 ^b	6.38 ^d
FWF 2.5%	7.42 ± 0.05 ^a	27.28 ± 0.68 ^{ab}	1.62 ± 0.02 ^b	2.17 ± 0.01 ^b	ND	61.51 ± 0.87 ^b	4.06 ± 0.11 ^b	5.73 ^e
FWF 5%	7.49 ± 0.06 ^a	27.18 ± 0.32 ^{ab}	2.71 ± 0.02 ^a	1.06 ± 0.02 ^e	ND	61.56 ± 0.65 ^b	4.40 ± 0.09 ^a	5.47 ^f
<i>F</i> -value	0.122	1.814	26,473	25,070	/	4.946	27.69	525,664
<i>P</i> -value	0.985#	0.185#	**	**	/	*	**	**

Legend: TTA: total titratable acidity; FWF: fermented wheat flour; PS: potassium sorbate; ND: not detected. *: $P < 0.05$; **: $P < 0.001$; #: statistically not significant. Means within the same column sharing a common letter are not significantly different ($P > 0.05$).



Table 4. Results of quantitative descriptive analysis of biscuits with added FWF and PS

	Reference	PS 0.2%	FWF 0.65%	FWF 1.25%	FWF 2.5%	FWF 5%	<i>F</i> -value	<i>P</i> -value
Odour	8.63 ± 0.52 ^a	7.75 ± 0.71 ^b	8.63 ± 0.52 ^a	8.50 ± 0.53 ^a	7.63 ± 0.52 ^b	4.88 ± 0.83 ^c	43.4	**
Taste	8.75 ± 0.46 ^a	7.63 ± 0.74 ^c	8.50 ± 0.53 ^{ab}	7.88 ± 0.83 ^{bc}	7.38 ± 0.92 ^c	5.63 ± 0.92 ^d	17.2	**
Flavour	8.63 ± 0.52 ^a	7.75 ± 0.46 ^b	8.13 ± 0.35 ^{ab}	8.00 ± 0.76 ^{ab}	6.88 ± 0.83 ^c	4.75 ± 0.71 ^d	39.6	**
Texture	8.88 ± 0.35 ^a	8.63 ± 0.52 ^{ab}	8.75 ± 0.46 ^a	8.13 ± 0.64 ^b	8.13 ± 0.64 ^b	7.50 ± 0.76 ^c	6.4	**

FWF: fermented wheat flour; PS: potassium sorbate; *: $P < 0.05$; **: $P < 0.001$. Values in a row followed by a different superscript letter are significantly different in the Duncan ($P < 0.05$) test.



Table 5. Results of hedonic test of biscuits with added FWF and PS

		Reference	PS 0.2%	FWF 0.65%	FWF 1.25%	FWF 2.5%	FWF 5%	F-value	P-value
Odour	Overall intensity	6.19 ± 1.94 ^{ab}	5.42 ± 2.06 ^b	6.35 ± 1.74 ^{ab}	6.96 ± 1.89 ^a	5.88 ± 1.90 ^{ab}	6.04 ± 2.37 ^{ab}	1.71	0.135#
	Margarine	5.46 ± 2.47 ^a	4.85 ± 2.57 ^a	4.27 ± 2.43 ^{ab}	4.15 ± 2.71 ^{ab}	4.23 ± 2.61 ^{ab}	3.19 ± 1.94 ^b	1.71	0.135#
	Burned	2.35 ± 1.89 ^{cd}	2.00 ± 1.94 ^d	3.58 ± 2.37 ^{bc}	4.42 ± 2.66 ^b	4.96 ± 2.59 ^{ab}	5.85 ± 2.82 ^a	10.15	**
	Fermented	2.19 ± 1.69 ^b	2.92 ± 2.39 ^b	4.50 ± 2.80 ^a	4.58 ± 2.73 ^a	5.08 ± 2.51 ^a	5.08 ± 2.13 ^a	6.55	**
Taste	Sweet	6.38 ± 1.57 ^a	5.65 ± 1.76 ^{ab}	5.69 ± 1.72 ^{ab}	5.42 ± 2.08 ^{ab}	4.96 ± 1.93 ^{bc}	4.04 ± 2.39 ^c	4.41	**
	Bitter	2.23 ± 1.66 ^d	2.04 ± 1.48 ^d	2.85 ± 1.18 ^{cd}	3.88 ± 2.49 ^{bc}	4.35 ± 2.21 ^b	6.04 ± 2.44 ^a	14.22	**
	Wet	2.31 ± 1.59 ^b	2.04 ± 1.28 ^b	2.73 ± 1.93 ^{ab}	3.12 ± 2.27 ^{ab}	3.58 ± 1.96 ^a	3.85 ± 2.59 ^a	3.32	*
Flavour	Overall intensity	5.96 ± 1.75 ^{ab}	5.19 ± 1.58 ^b	5.62 ± 1.65 ^{ab}	6.31 ± 1.96 ^{ab}	5.85 ± 1.78 ^{ab}	6.50 ± 2.27 ^a	1.70	0.137#
	Margarine	5.85 ± 2.38 ^a	5.08 ± 1.99 ^{ab}	4.42 ± 1.98 ^b	4.50 ± 2.28 ^b	4.54 ± 2.55 ^b	3.88 ± 2.05 ^b	2.39	*
	Sour/fermented	2.38 ± 1.90 ^c	2.50 ± 1.90 ^c	3.58 ± 2.56 ^{bc}	4.31 ± 2.49 ^b	4.15 ± 2.43 ^b	5.73 ± 2.65 ^a	7.44	**
Texture	Crumbliness	6.85 ± 1.59 ^{ab}	6.23 ± 1.88 ^b	6.62 ± 1.94 ^{ab}	6.96 ± 1.68 ^{ab}	6.65 ± 1.94 ^{ab}	7.58 ± 1.88 ^a	1.58	0.170#
	Crispiness	7.46 ± 1.61 ^a	7.62 ± 1.27 ^a	7.15 ± 1.67 ^a	7.12 ± 1.88 ^a	7.00 ± 1.57 ^a	5.88 ± 2.57 ^b	2.97	0.0138#
	Mouth coating	5.54 ± 2.30 ^a	5.35 ± 1.98 ^a	4.92 ± 1.87 ^a	5.65 ± 2.11 ^a	4.92 ± 2.04 ^a	5.92 ± 2.04 ^a	0.99	0.4230#

FWF: fermented wheat flour; PS: potassium sorbate; *: $P < 0.05$; **: $P < 0.001$; #: statistically not significant. Values in a row followed by a different superscript letter are significantly different in the Duncan test ($P < 0.05$).



crumbliness and mouth-coating characteristics. The obtained results clearly show that adding FWF to biscuits significantly affects the burned and fermented odour notes. The results also indicate that there are no significant differences in mouth-coating among samples.

4. CONCLUSIONS

This study has found that FWF contains organic acids (e.g. acetic acid, lactic acid and pyruvic acid). Moreover, we have demonstrated the antibacterial potential of FWF against *S. aureus*, *B. cereus* and *B. subtilis* and that FWF can preserve food as well as synthetic PS. Secondly, the study has shown that adding FWF to biscuits results in increased ash content, TTA and decreased pH. Furthermore, the addition of FWF in the recommended concentration range between 0.65% and 2.5% has no negative effect on overall sensory characteristics. Therefore, FWF represents a good candidate for a PS preservative substitute in the bakery industry.

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