

LYOPHILIZED KALE (*BRASSICA OLERACEA* L.) LEAVES AS AN ENHANCING COMPONENT IN GLUTEN-FREE BREAD

– Research paper –

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Abstract: The objective of the research was to assess the impact of powdered kale (*Brassica oleracea* L. var. *acephala*) on the properties of starchy gluten-free dough and bread. Kale lyophilisate was added to the recipe as 3%, 6%, or 9%, replacing the starch. The addition of the kale resulted in a decrease in the dough's strength despite the optimisation of the water content. The presence of kale lyophilisate reduced the volume of the loaves, in comparison with control, resulting in a decrease in crumb porosity and pore density, and an increase in the quantity of large pores. The addition of lyophilised kale at all levels resulted in an increase in taste acceptability, while the 6 and 9% kale decreased the acceptability of the other sensory parameters. The presence of the kale lyophilisate led to a reduction in crumb hardness on individual days of storage compared to the control sample, and the decrease was greater the more kale powder was added to the recipe. Bread with the highest concentration of the kale lyophilisate showed lower enthalpy of retrograded amylopectin decomposition in subsequent days of storage.

Keywords: kale leaves; starchy dough; bread quality; crumb staling; rheology; texture

INTRODUCTION

Kale (*Brassica oleracea* L. var. *acephala*) is a green leafy vegetable belonging to the cabbage family (*Brassicaceae*), which also includes cabbage, cauliflower, broccoli, and Brussels sprouts. In recent years, kale has become particularly popular and is gaining more and more recognition among consumers as a “superfood”. It holds a significant position in people's nutritional and culinary diets in many regions, mainly in Asia, Europe, and America. It also appears on the menus of many restaurants promoting healthy eating (Šamec et al., 2019). The popularity of kale is due to its interesting organoleptic qualities and, undoubtedly, its high nutritional value. Many authors emphasize the significant amount of bioactive compounds in kale, such as pro-vitamin A, vitamin C, glucosinolates, phenolics or dietary fibre (Agarwal et al., 2017; Becerra-Moreno et al., 2014; Emebu & Anyika, 2010). In addition, in the

category of green leafy vegetables, kale is a notable source of essential minerals including phosphorus, calcium, iron, magnesium, sodium, zinc, and potassium, which play an important role in metabolic processes (A. Korus, 2020). (Di Noia, 2014).

The World Health Organization (WHO) recommends eating at least 400 g of non-starchy vegetables and fruits (World Health Organization, 2020). According to the Dietary Guidelines for Americans, the recommended daily intake of vegetables is five servings, based on a 2,000 calorie diet (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). One of the five servings of vegetables should be green leafy vegetables. According to Carter et al. (2010) and Bhupathiraju et al. (2013), the consumption of dark green leafy vegetables is linked to a lower risk of developing diabetes and heart disease. Both *in vitro* and *in vivo* studies indicate that kale exhibits a beneficial impact on the prevention of chronic, particularly cardiovascular, diseases (Kahlon et al., 2007; Kim

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et al., 2008; Kural et al., 2011) and cancer (Royston & Tollefsbol, 2015). Kim et al. (2008) showed that regular consumption of kale juice may have a beneficial effect on the blood cholesterol and triglycerides levels and antioxidant defense system, and thus can help lower the risk of coronary heart disease among men suffering from hyperlipidemia. Therefore, meeting the expectations of consumers who care about a healthy diet and are looking for new products with increased nutritional value, an interesting solution may be to enrich food with the addition of the kale lyophilisate. For example, the addition of the kale lyophilisate to milk chocolates made it possible to modify their physical, nutritional, and physicochemical properties. Enriched chocolates showed higher fibre and mineral content, and thus a higher nutritional value (Carvalho et al., 2018). Kasprzak et al. (2018), after enriching corn snacks with kale, observed a high level of phenols in the

products. According to these authors, kale, as a raw material with high antioxidant potential, can be a valuable source of natural antioxidants. In an earlier study, we demonstrated that incorporating freeze-dried kale elevates the levels of dietary fibre, chlorophylls, and carotenoids, but significantly affects various characteristics of dough and wheat bread (A. Korus et al., 2023). Klopsch et al. (2019) studying the effect of adding 7-day microgreens and 14-day-old kale leaves to wheat bread on the content of secondary plant metabolites, they found an increase in the amount of certain bioactive compounds in bread. Therefore, it seems advisable to investigate the impact of incorporating kale leaves on the quality of gluten-free bread, which due to its specific composition exhibits a lower nutritional value when compared to wheat bread and a much weaker structure.

MATERIALS AND METHODS

Materials

The research material was maize starch (Bezgluten, Poland) and potato starch (Pepees SA, Poland), pectin (Pektowin, Poland), guar gum (Lotus Gums & Chemicals, India), lyophilised yeast (Lesaffre, France), salt, sugar, rapeseed oil (purchased from a local supermarket) and water. Kale lyophilisate powder with the following composition was used as an enriching ingredient in the gluten-free bread recipe: total dietary fibre 23.56 ± 0.21 g/100g, soluble dietary fibre 5.27 ± 0.08 g/100g, starch 12.07 ± 0.25 g/100g, sugars 11.39 ± 0.39 g/100g, ash 7.50 ± 0.11 g/100g, polyphenols 1236 ± 16 mg/100g, chlorophylls (a+b) 766.7 ± 12.7 mg/100g, carotenoids 205.0 ± 7.4 mg/100g. Fresh retail kale leaves were blanched in 98°C water for 2.5 minutes, and then frozen in the trays of a lyophiliser in the deflector chamber of Feutron GmbH (model number 3626-51). The freezing process was conducted at -40°C for 90 minutes. A CHRIST Gamma 1-16 LSC freeze dryer (Shrewsbury, UK) was used to dry kale leaves. The lyophilisation drying process began at an initial temperature of -25°C. The dried material with a water content of about 5% was obtained after 30 hours of drying. Then, the lyophilisate was ground (GM200 mill, Retsch, Germany), sieved and then stored in airtight glass jars.

Bread recipes

The dough used to bake control gluten-free bread (GFB) was obtained from the following

components: maize starch 560 g, potato starch 140 g, lyophilised yeast 35 g, vegetable oil 21 g, saccharose 14 g, salt 11.7 g, pectin 11.7 g, guar gum 11.7 g and water 694 g. In supplemented breads (KP) a portion of both starches was substituted with kale leaf lyophilisate in the amount of 3%, 6%, or 9%. Due to the significant effect of the kale lyophilisate on the increase in the water absorption of the dough, the samples with its addition contained larger amounts of water, 735, 773, and 816 g, respectively. The water amount was established through the method of back extrusion using a TA-XT2plus texture analyser (Stable Micro Systems, UK).

Dough texture and its rheological properties

The rheological features of the dough were assessed at $25 \pm 0.5^\circ\text{C}$ using a MARS II rheometer (ThermoFisher Scientific, USA) outfitted with a parallel serrated plates system (diameter 35 mm, gap size 1 mm). The dough samples were positioned within the rheometer's measuring system, left to relax for 5 minutes to alleviate any stress and normalize the temperature, and protected from evaporation using paraffin oil.

Mechanical spectra were established within the linear viscoelastic range, utilizing a consistent strain amplitude of 0.1%, in the frequency range of 1-100 rad/s. Empirical data was expressed through power equations:

$$G'(\omega) = K' \cdot \omega^{n'} \quad (1)$$

$$G''(\omega) = K'' \cdot \omega^{n''} \quad (2)$$

where: G' – storage module (Pa), G'' - loss module (Pa), ω - angular frequency (rad/s), K' , K'' , n' , n'' - constants.

Creep and recovery tests were conducted under a constant deformation conditions in the creep phase $\tau_0 = 2$ Pa for 150 s. The duration of the recovery phase was 300 seconds. The acquired results were depicted through the implementation of the Burgers model:

$$J(t) = J_0 + \frac{t}{\eta_0} + J_1 \cdot (1 - \exp^{-t/\lambda_{ret}}) \quad \text{for the creep phase} \quad (3)$$

$$J(t) = \frac{t_1}{\eta_0} - J_1 \cdot (1 - \exp^{t_1/\lambda_{ret}}) \cdot \exp^{-t/\lambda_{ret}} \quad \text{for the recovery phase} \quad (4)$$

where: J – compliance (Pa^{-1}), J_0 – instantaneous compliance (Pa^{-1}), J_1 – viscoelastic compliance (Pa^{-1}), η_0 – zero shear viscosity ($\text{Pa}\cdot\text{s}$), λ_{ret} – retardation time (s), t_1 – time after which the stress was removed (s).

The gluten-free dough textural properties were determined according to the methodology described by Ren et al. (2020) using a TA-XT2plus texture analyser (Stable Micro Systems, England). 100 g of dough samples were placed in a container (height 7 cm, internal diameter 5 cm) and extruded at 1 mm/s with a plastic disc 3.5 cm in diameter and 5 mm thick to a penetration depth of 22 mm. Subsequently, the measuring element was retracted at a speed of 1 mm/s. Based on the back extrusion curves in the time-force system, the dough's firmness and consistency were reflected by the maximum positive force and corresponding area during extrusion. Conversely, the maximum negative force and corresponding area during the retraction of the measurement element reflected the dough's cohesiveness and viscosity index.

Preparation of the dough and baking of the bread

The dough was prepared by mixing the ingredients for 8 minutes using a spiral mixer (SP 12, Diosna, Germany). Next, the dough was fermented for 15 minutes at a temperature of 35°C, followed by an additional minute of mixing. The dough was divided into 250 g pieces and inserted into metal molds. The final fermentation process occurred for 20 minutes under identical conditions. Bread baking was performed for 30 minutes at 230°C using an electric oven (CO 2 0608, MIWE GmbH, Germany) and then loaves were allowed to cool prior to analyses. Samples were subject to storage at a controlled temperature of 20±2°C within polyethylene bags to monitor changes in texture over time.

Bread analyses

The loaves' volume was quantified with a Volscan 600 (Stable Micro Systems, UK). One-centimeter-thick samples obtained from the inside of each loaf underwent image analysis using a desktop scanner. ImageJ v. 1.53m software was used to analyze the images and measure the porosity, pore density, and average pore size.

The analysis of the colour of the crumb in the CIE $L^*a^*b^*$ system was carried out using the reflection method with the Color i5 spectrometer (X-Rite, USA) measuring geometry d/8, light source D65, and observer 10°. The total colour difference (ΔE) from the control sample was also calculated.

The texture profile analysis (TPA) of the bread crumb was carried out using the TA-XT2plus texture analyser (Stable Micro Systems, Great Britain), and the obtained measurement data were processed with the Texture Exponent program (Stable Micro Systems, Great Britain). Using a P/35 aluminium cylindrical probe, a 2 cm high crumb sample was compressed with a 50% deformation rate in two cycles at a compression rate of 5 mm/s. The measurements were conducted on the baking day and for two consecutive days.

The thermal characterisation of the bread crumb was performed using the DSC 204F1 Phoenix differential scanning calorimeter (Netzsch GmbH, Germany) and the Proteus Analysis program (Netzsch GmbH, Germany). To ascertain the freezable-water content (Ribotta & Le Bail, 2007), crumb samples weighing about 15 mg were sealed in aluminium vessels and cooled from 20 to -60°C at a rate of 10°C/min. After being held at this temperature for 5 minutes, they were heated to 50°C at a rate of 10°C/min. An empty aluminium dish served as the reference. Freezable-water content was reported in g/g.

On each day of the analysis, crumb samples weighing about 15 mg were taken from the center of the loaf, hermetically sealed in aluminium dishes, and heated in a calorimeter from 20 to 100°C at a rate of 10°C/min. An empty aluminium dish was used as a standard. Enthalpy values are expressed in J/g of d.b.

The bread acceptance analysis was conducted by a team of 14 evaluators. The analysis of the acceptance of individual features of the labeled with specific codes bread samples was performed on a seven-point scale (1 means “I don’t like”, 7 means “I like very much”). The analysis included qualitative characteristics such as general appearance, structure, porosity, colour, smell, and taste.

Statistical analysis

The data were analyzed using one- and two-way ANOVA and the Duncan test was employed to determine significant differences between means at a 0.05 significance level. Pearson's correlation

coefficients were calculated to evaluate correlations between parameters using the Student's t-test for significance. Statistica 12.0 software (StatSoft Inc., USA) was utilized for calculations and analysis.

RESULTS AND DISCUSSION

Dough texture and its rheological properties

The storage module (G') was found to be greater than the loss module (G'') in all tested doughs, indicating a prevalence of elastic features (Figure 1a). Simultaneously, a statistical correlation between the modules and the angular frequency was noticed, which is further supported by the values of tangent values of the phase shift angle within the $0.1 < \tan \delta < 1.0$ range (Figure 1b). This suggests that the doughs under examination exhibit characteristics aligned with the formation of weak gels, supporting previous findings on the rheological attributes of doughs free of gluten (J. Korus et al., 2021; M. Witczak et al., 2019). The presence of the kale lyophilisate affected the change in the values of the G' and G'' modules of the dough in different ways (Figure 1a). The inclusion of the lowest concentration led to a noticeable reduction in module values. Alternatively, the larger concentration of the kale lyophilisate had a comparatively minor impact on the alterations in moduli values. Small changes in

the module values may result from the need to optimize the amount of water in the recipe. The inclusion of kale lyophilisate in the dough recipe led to an elevation in the $\tan \delta$ value (Figure 1b), particularly at frequencies below 10 rad/s, implying a reduction in the structural integrity of the dough.

These observations confirm the values of the power model parameters, which depict the relationship between the G' and G'' modules and the angular frequency (Table 1). The value of the parameter K' , which indicates the initial value of the G' module, decreased significantly ($p < 0.05$) for the lowest concentration addition of the kale lyophilisate, while its higher addition did not exert a significant ($p > 0.05$) influence on this parameter. In comparison to the control sample, the n' parameter values were higher (although the result was not significant at $p > 0.05$) (Table 1), suggesting a reduction in the modules' reliance on the angular frequency.

Table 1. Rheological characteristics of the control gluten-free dough (GFD) and samples containing kale powder (KP) supplement.

Parameter	Control GFD	KP 3%	KP 6%	KP 9%	ANOVA - p
K' (Pa·s ^{n'})	1473.6±61.5 ^b	1141.0±138.3 ^a	1461.1±64.6 ^b	1318.0±86.5 ^b	0.008
n'	0.203±0.001 ^a	0.219±0.012 ^b	0.223±0.011 ^b	0.225±0.004 ^b	0.044
$R^2 >$	0.998	0.994	0.995	0.998	
K'' (Pa·s ^{n''})	548.5±23.0	534.4±91.6	645.2±30.4	582.3±38.7	0.127
n''	0.196±0.001	0.201±0.010	0.189±0.002	0.192±0.004	0.148
$R^2 >$	0.970	0.978	0.970	0.974	
$\tan \delta$ at 1 rad·s ⁻¹	0.416±0.004 ^a	0.456±0.017 ^{ab}	0.493±0.040 ^b	0.492±0.002 ^b	0.007
$J_0 \cdot 10^{-3}$ (Pa ⁻¹)	1.21±0.00005	1.35±0.00014	1.38±0.00004	1.48±0.00002	0.056
$J_1 \cdot 10^{-3}$ (Pa ⁻¹)	1.89±0.0008 ^a	2.98±0.00001 ^b	2.61±0.00020 ^b	2.67±0.00042 ^b	0.023
η_0 (kPa·s)	39.16±0.11 ^b	27.35±2.45 ^a	26.29±3.25 ^a	25.79±3.60 ^a	0.013
λ_{ret} (s)	65.0±0.1	73.9±0.8	71.6±3.2	77.6±8.4	0.130
$R^2 >$	0.993	0.980	0.994	0.993	
Firmness N	12.62±0.61 ^c	9.56±0.81 ^a	9.59±0.64 ^a	10.83±0.29 ^b	0.001
Consistency N·s	101.8±6.68 ^b	80.8±7.40 ^{ab}	83.6±9.48 ^a	88.9±3.1 ^a	0.027
Cohesiveness N	-9.06±0.74 ^a	-7.25±0.53 ^{ab}	-7.47±0.48 ^b	-8.19±0.20 ^b	0.011
Index of Viscosity N·s	-80.5±5.1	-64.2±7.5	-67.4±9.6	-69.1±4.0	0.086

Mean values ± standard deviation. The mean values with identical letters in specific rows are not significantly different at a 0.05 level of confidence

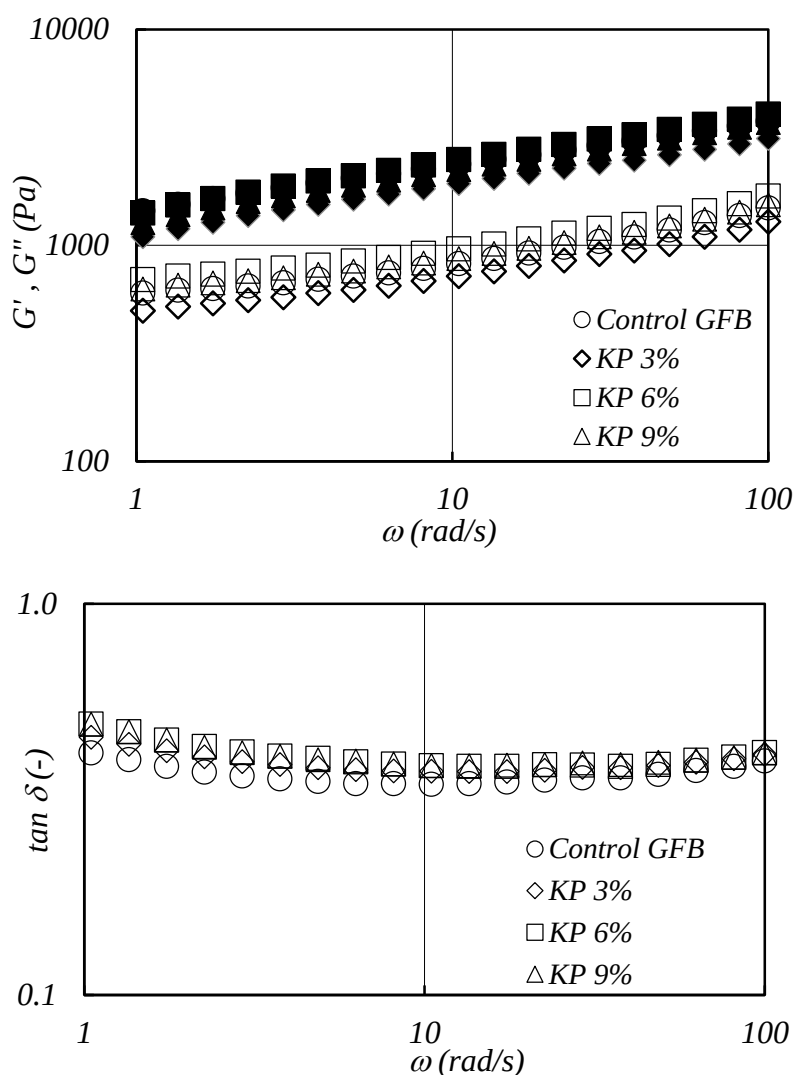


Figure 1. Rheological curves of control dough and dough with kale powder addition: a) sweep frequency, b) tangent of shift angle ($\tan \delta = G''/G'$); G' - filled symbols, G'' - empty symbols

The concentration of the kale lyophilisate in the recipe did not have a statistical effect ($p > 0.05$) on the values K'' and n'' describing the impact of angular frequency on the G'' values (Table 1). Conversely, at an angular frequency of 1 rad/s, there was a notable rise in the values of $\tan \delta$ for the 6% and 9% concentrations, signifying a deterioration in the structural integrity of the dough, indicating a weakening of the dough structure (Table 1), which confirms the course of the curves shown in Figure 1b.

Figure 2 shows that the control dough exhibited noticeably reduced compliance values when compared to the kale-contained samples. The incorporation of kale lyophilisate in the recipe led to a significant increase in the dough's sensitivity to applied stress, although the concentration of the kale did not have a significant effect. The kale lyophilisate had no significant impact ($p > 0.05$) on the instantaneous compliance J_0 values. Conversely, it had a significant ($p < 0.05$) impact

on the rise in the viscoelastic compliance value J_1 , in comparison to the control dough. However, the concentration of the kale did not have any significant impact. Similarly, for zero shear viscosity η_0 , the presence of the kale lyophilisate affected their reduction compared with the control dough, but the concentration of the kale itself had no significant ($p > 0.05$) impact. Nevertheless, there was no statistical ($p > 0.05$) disparity in the retardation time (λ_{ret}) values, which correspond to the duration needed to increase the strain value during the creep phase up to 63% of the total value.

In terms of large deformations, incorporating kale lyophilisate led to a reduction in firmness, consistency, and cohesion in comparison with the control dough, indicating a weakening of the dough structure (Table 1). However, the observed changes in viscosity index values were not statistically significant ($p > 0.05$).

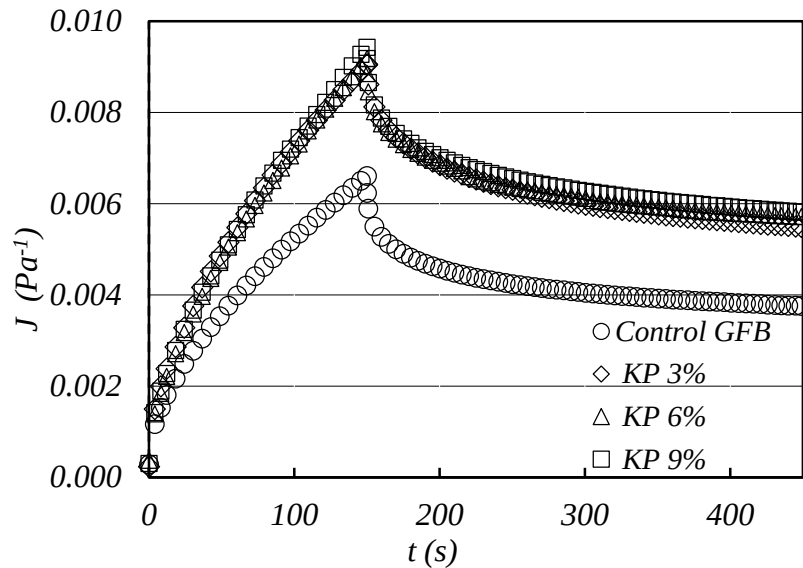


Figure 2. Curves illustrating creep and recovery in gluten-free dough samples, including the control and those with added kale powder

The results obtained while evaluating the rheological characteristics of the dough allow to conclude that the addition of the kale lyophilisate to the recipe weakens the dough structure, despite the simultaneous optimisation of the addition of water. This is confirmed by an increase in the tangent of the phase shift angle and instantaneous compliance, as well as a decrease in zero shear viscosity, cohesion, and consistency. The primary factor affecting the variations in rheological characteristics seems to be the water absorption of the components. Wang et al. (2020) observed an increase in water absorption, a weakening of the dough structure and a decrease in its viscosity caused by the addition of celery powder. The gluten-free dough's structure is created and stabilized, in this case, by swollen starch granules filling the spatial network of hydrocolloids, in this case namely, guar gum and pectin. The addition of powdered kale lyophilisate with high water absorption into the system causes it to compete

with starch granules for water molecules, limiting their swelling, which in turn causes looser packing and the presence of empty spaces, thus weakening the structure of the dough.

Bread quality

The concentration of the kale lyophilisate at 3% or 6% significantly ($p < 0.05$) reduced the volume as well as the specific volume of the obtained bread compared to the control sample (Table 2). The largest 9% concentration of the kale significantly ($p < 0.05$) increased in the discussed parameters compared to the enriched samples; however, these values were still much lower relative to the control bread. Confirmation of the discussed changes are the observations of cross-sections of the surface of the loaves shown in Figure 3. Similarly, Wang et al. (2020) noticed a reduction in the specific volume of bread loaves containing powdered celery.

Table 2. Physical and colour characteristics of control bread (GFB) and loaves of bread containing kale powder (KP) addition

Parameter	Control GFB	KP 3%	KP 6%	KP 9%	ANOVA - p
Volume (cm ³)	670±10.0 ^c	568±8.4 ^a	574±8.9 ^a	598±4.5 ^b	<0.001
Specific volume (cm ³ ·g ⁻¹)	3.24±0.05 ^c	2.77±0.04 ^a	2.78±0.05 ^a	2.95±0.02 ^b	<0.001
Porosity	0.510±0.000 ^b	0.493±0.006 ^a	0.490±0.000 ^a	0.487±0.006 ^a	0.001
Cell density (cm ⁻²)	17.6±0.4 ^d	16.1±0.1 ^c	13.5±0.3 ^b	11.3±0.3 ^a	<0.001
Percentage of pores>5mm ²	17.3±0.3 ^a	18.7±0.5 ^b	21.0±0.8 ^c	23.1±0.6 ^d	<0.001
L^*	74.6±0.32 ^d	60.1±0.09 ^c	47.4±0.20 ^b	41.5±0.31 ^a	<0.001
a^*	-1.03±0.09 ^c	-2.15±0.02 ^a	-1.10±0.05 ^c	-1.32±0.05 ^b	<0.001
b^*	15.4±0.27 ^a	21.4±0.07 ^b	24.4±0.11 ^d	23.8±0.12 ^c	<0.001
ΔE	-	15.8±0.11	28.6±0.23	34.2±0.34	

Mean values ± standard deviation. The mean values with identical letters in specific rows are not significantly different at a 0.05 level of confidence

The kale powder contains different compounds that can influence the characteristics of dough or bread in varying manners. The addition of high-molecular carbohydrates forming fibre fractions into the system will increase water absorption, which will increase the yield of the dough and reduce the rate of bread staling.

Although no significant differences were noted between the concentrations of the additive ($p > 0.05$), the substitution of a portion of flour with kale powder caused a reduction in the porosity of the crumb relative to the control sample. On the other hand, a distinct impact resulting from the inclusion of kale lyophilisate was observed in the case of pore density, which decreased significantly ($p < 0.05$) according to increasing concentration, and the presence of large pores ($>5 \text{ mm}^2$), the values of which significantly ($p < 0.05$) increased with the increase in kale concentration. These findings support the observed decrease in the bread volume and suggest a potential weakening of the structural integrity of the dough, leading to a reduced capacity to retain fermentation gases., resulting in the formation of larger gas bubbles and their smaller number (Table 2). The discussed results are supported by the images of the bread cross-section shown in Figure 3. As the concentration of kale lyophilisate in the recipe rises, a notable augmentation in pore size and a reduction in their density per unit area become apparent.

Starch bread, due to its poor composition, is characterized by a light colour (J. Korus et al., 2021; T. Witczak et al., 2017). The addition of the kale powder caused a significant darkening of the crumb and an increase in the value of the

parameter b^* , indicating the share of yellow colour with a low value of the a^* parameter, which determines the share of green colour (Table 2). As a green vegetable, kale is rich in chlorophyll, which significantly modifies the colour of the bread enriched. Likewise, Drabińska et al. (2018) observed that the addition of a powdered preparation based on broccoli to the recipe of gluten-free cookies causes a change in their colour and a significant increase in the share of green colour. The total colour difference values (ΔE) indicate significant differences in the colour of the crumb relative to the control bread, and the more lyophilisate was added to the recipe, the greater these values. These results are confirmed by the photographs of bread (Figure 3). The smallest 3% concentration of the kale powder clearly reduced the brightness of the crumb; however, the green shade is imperceptible. In the case of samples with 6% and 9% addition, the bread crumb is clearly darker, and the green-yellow colour is definitely noticeable. Krupa-Kozak et al. (2021) also discovered that the use of a broccoli preparation in gluten-free bread resulted in a noticeably darker colour of the crumb and crust. The gluten-free bread containing a broccoli preparation showed a significant increase in green and yellow tones, with significantly reduced negative values for the a^* parameter and significantly increased values for the b^* parameter. These findings, along with previous literature, provide clear evidence that incorporating vegetable raw materials or industrial waste as ingredients in bakery products can significantly impact their colour (Majzoobi et al., 2016), which is not always accepted by consumers.

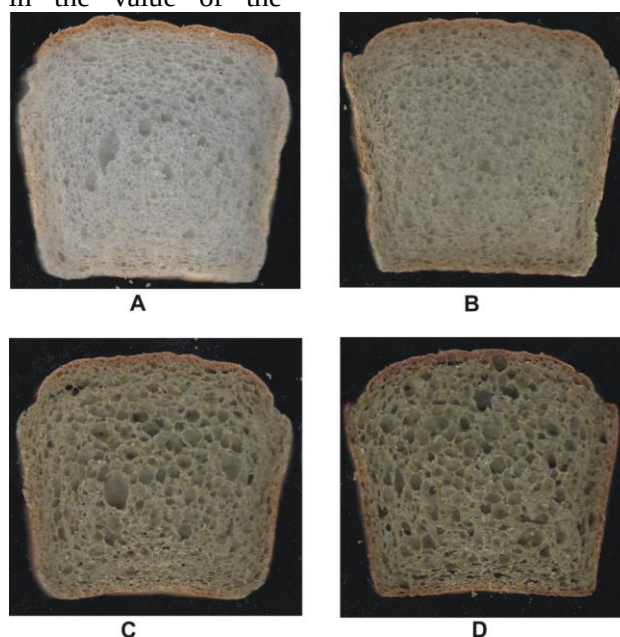


Figure 3. Visual representation of gluten-free bread: (a) represents the control bread, while (b), (c), and (d) depict bread supplemented with kale flour at 3%, 6%, and 9% concentrations respectively.

The smallest 3% concentration of the kale powder had no significant ($p > 0.05$) effect on the acceptability of external appearance, structure and porosity, crumb colour and smell (Figure 4). This is supported by the observations of the bread images shown in Figure 3, where it can be observed that the structure and appearance of bread with 3% of kale powder is similar to the control sample, although the crumb of this bread is slightly darker in colour (Table 2). It was found, however, that the lowest 3% concentration of the kale lyophilisate positively affects the acceptability of the taste, which may be because additional ingredients are added, which during thermal processing create substances that favorably enrich the taste. For bread recipes containing 6% and 9% concentrations of kale powder, sensory parameters, except for taste, showed a decrease in their acceptability. This is supported by the observations of the bread images (Figure 3), where the image of

the structure is characterized by the presence of large pores and a significant change in colour (Table 2), which, due to the green tint resulting from the presence of chlorophyll, may not be associated by consumers with the typical colour of bread.

In the case of palatability, only 9% concentration of the kale lyophilisate led to a significant ($p < 0.05$) decrease in the acceptability of this feature. Wang et al. (2020) reported a reduction in the acceptability of bread incorporating powdered celery in terms of sensory characteristics, including colour, texture, taste, and aroma, as well as overall acceptability. In their study, Drabińska et al. (2018) found that adding a powdered preparation based on broccoli to the recipe of gluten-free cookies resulted in a substantial decrease in the product's acceptability. This was attributed to its green colour, increased hardness, and intense aroma and flavour.

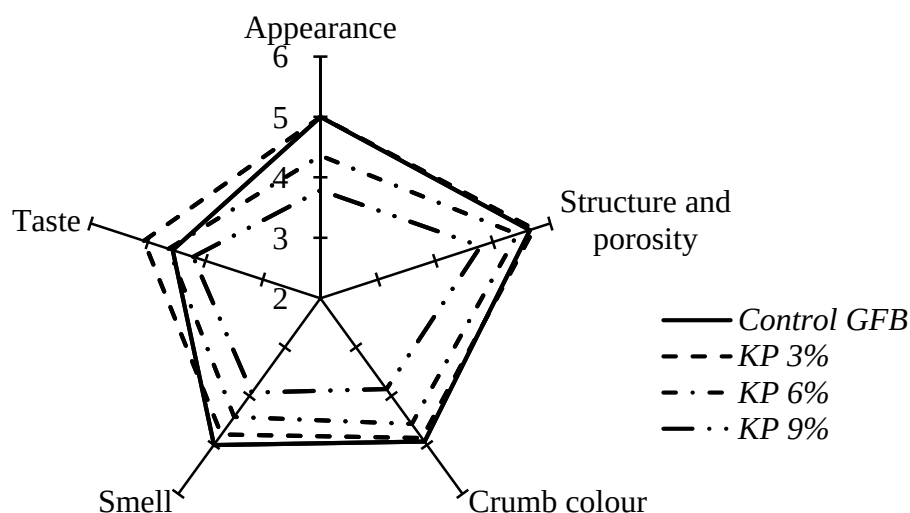


Figure 4. The outcomes of sensory evaluation conducted on control gluten-free bread and samples with varying amounts of kale flour addition

On the first day, the control crumb exhibited a hardness of 0.8 N (Table 3). Throughout the storage period, there was a significant ($p < 0.05$) increase in the hardness of the crumb, reaching over 4 N, which confirms our earlier research (J. Korus et al., 2021). Incorporating the kale lyophilisate in the recipe led to a reduction in crumb hardness on the baking day, but it was not statistically significant ($p > 0.05$). In contrast, Wang et al. (2020) reported an elevation in crumb hardness of wheat bread fortified with powdered celery. An increase in crumb hardness was observed by Ranawana et al. (2016) on bread enriched with powdered vegetables of various origins. During the storage of the tested bread, its hardness on individual days was lower in relation

to the control bread and, in general, the decrease in hardness was greater the more the kale powder was added to the recipe. The two-factor analysis of variance revealed that both the concentration of kale and storage time, as well as their interaction, had a significant ($p < 0.05$) influence on crumb hardness. Whereas Krupa-Kozak et al. (2021) did not show a significant effect of enriching gluten-free bread with powdered broccoli on their crumb hardness during storage. On the first two days of testing, there was no significant ($p > 0.05$) differentiation in the crumb springiness of the tested bread. Only on the third day after baking, a significant decrease ($p < 0.05$) in the springiness of the crumb of the samples with 6% and 9% concentration of the kale lyophilisate was observed

in relation to the control. Such significant decrease in the springiness of the crumb samples with a higher concentration of the kale powder may be related to its structure characterized by a lower pore density and a greater number of large pores (Table 2, Figure 3). Wang et al. (2020) reported a downward trend in crumb elasticity of breads enriched with celery powder. It was noted that the content of the kale lyophilisate as well as time of storage had a significant ($p < 0.05$) effect on crumb springiness, while the combination of these two factors did not ($p > 0.05$). Another parameter characterizing the texture of the crumb is its cohesiveness. This feature is also important for

consumers and is associated with the tendency to crumbling of crumb with increasing storage time. The obtained results indicate that the concentration of the kale lyophilisate, time of storage, and interaction of these factors have a significant ($p < 0.05$) effect on the cohesiveness values, which in most cases decreased compared to the control sample (Table 3). Whereas Krupa-Kozak et al. (2021) showed greater crumb cohesiveness of GFB containing broccoli powder during storage. Since the chewiness of the crumb is closely associated with hardness, the modifications arising from the utilization of kale lyophilisate, and storage time had a similar trend (Table 3).

Table 3. Results of TPA analysis of control bread (GFB) and bread containing powdered kale lyophilisate (KP)

Sample	Day	Hardness (N)	Springiness (-)	Cohesiveness (-)	Chewiness(N)
	1	0.78±0.09 ^a	1.00±0.00 ^d	0.90±0.01 ^c	0.84±0.09 ^{ab}
Control GFB	2	4.22±0.19 ^d	0.98±0.01 ^{cd}	0.57±0.05 ^b	2.37±0.17 ^e
	3	4.16±0.20 ^d	0.98±0.01 ^{cd}	0.59±0.08 ^b	2.40±0.26 ^e
	1	0.69±0.07 ^a	1.00±0.00 ^d	0.90±0.02 ^c	0.69±0.14 ^a
KP 3%	2	2.63±0.20 ^{bc}	0.98±0.02 ^{cd}	0.61±0.03 ^b	1.58±0.20 ^d
	3	3.03±0.55 ^c	0.97±0.01 ^{bc}	0.55±0.05 ^b	1.62±0.45 ^d
	1	0.68±0.11 ^a	1.00±0.00 ^d	0.87±0.01 ^c	0.60±0.10 ^a
KP 6%	2	2.46±0.64 ^{bc}	0.98±0.02 ^{cd}	0.60±0.04 ^b	1.46±0.52 ^{cd}
	3	2.60±0.69 ^{bc}	0.95±0.03 ^{ab}	0.54±0.04 ^b	1.35±0.49 ^{bcd}
	1	0.82±0.26 ^a	1.00±0.00 ^d	0.86±0.01 ^c	0.71±0.22 ^a
KP 9%	2	2.29±0.34 ^b	0.96±0.01 ^{bc}	0.60±0.03 ^b	1.33±0.26 ^{bcd}
	3	2.42±0.30 ^{bc}	0.92±0.02 ^a	0.43±0.06 ^a	0.95±0.13 ^{abc}
Two-way ANOVA- <i>p</i>					
Factor A (level)		<0.001	0.008	0.020	<0.001
Factor B (time)		<0.001	<0.001	<0.001	<0.001
Factor A × Factor B		0.001	0.096	0.030	0.029

Mean value ± standard deviation. The mean values with identical letters in specific columns are not significantly different at a 0.05 level of confidence

The characteristic transformation parameters, including the onset temperature (T_o), peak temperature (T_p), and end temperature (T_E), were measured as -6.4°C, 4.1°C, and 12.3°C, respectively, for the control bread's crumb. (data not shown). The values of these parameters were not significantly affected by the presence of kale lyophilisate in the recipe. The determined values of the enthalpy of transformation related to the melting of ice crystals ranged from 103.2 J/g in the case of the GFB to 138.7 J/g for the KP 9% and showed a significant increase ($p < 0.05$) with the increasing amount of lyophilisate in the recipe. A higher range of values for this parameter is given by Ribotta and Le Bail (2007). The tested crumb samples contained from 30.9 to 41.5% of freezable water on the first day of storage (Figure 5), which is related to its higher addition due to the preservation of the dough's consistency with

increasing addition of the hygroscopic kale lyophilisate. As the statistical analysis showed, the concentration of the kale powder, and thus the increasing amount of added water, the time of storage, and combination of these factors had a statistical ($p < 0.05$) effect on the level of freezable-water. Generally, the content of freezable water decreased with the storage time of the bread. (Figure 5), however, these changes were not statistically significant ($p > 0.05$).

The T_o values ranged from 47.8 oC for the control sample to 50.1 oC for bread crumb with a 6% content of kale lyophilisate; however, the differences between the average values were not statistically significant (Table 4). With the increase in the storage time, the values of the T_o increased, especially for 6% and 9% concentration in the kale powder.

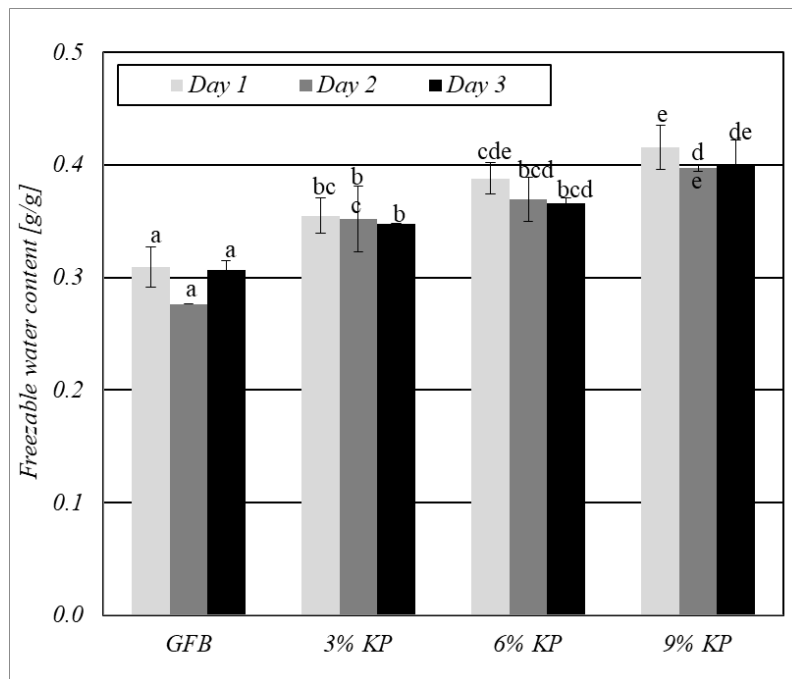


Figure 5. Freezable water content in control bread and samples containing kale powder

Table 4 Thermal transition parameter of retrograded amylopectin of control gluten-free bread (GFB) and samples with kale powder (KP) addition

Sample	Day	T _O (°C)	T _P (°C)	T _E (°C)	-ΔH (J·g d.b. ⁻¹)
Control GFB	1	47.80±0.00 ^a	65.70±0.28 ^{cd}	78.10±6.36	1.33±0.12 ^b
	2	52.85±1.20 ^{cde}	64.85±0.07 ^{bcd}	77.50±2.40	2.95±0.05 ^d
	3	51.00±0.14 ^{bcd}	64.00±0.42 ^{bc}	79.15±0.78	4.03±0.14 ^g
KP 3%	1	49.45±1.63 ^{ab}	63.70±0.14 ^b	82.00±0.57	1.01±0.14 ^{ab}
	2	51.50±0.14 ^{bcd}	64.05±0.07 ^{bc}	78.25±0.35	3.42±0.09 ^{ef}
	3	51.95±0.21 ^{bcd}	64.20±0.00 ^{bc}	78.65±0.49	3.77±0.52 ^{fg}
KP 6%	1	50.05±2.76 ^{abc}	63.95±2.05 ^{bc}	80.55±3.18	1.46±0.26 ^b
	2	53.70±1.84 ^{de}	65.70±1.27 ^{cd}	76.60±1.27	3.10±0.13 ^{de}
	3	53.10±1.56 ^{cde}	64.65±0.07 ^{bcd}	79.50±0.14	3.61±0.08 ^{fg}
KP 9%	1	49.95±0.92 ^{abc}	61.50±0.57 ^a	70.75±1.34	0.62±0.08 ^a
	2	53.60±1.56 ^{de}	65.45±0.21 ^{bcd}	77.25±1.63	2.26±0.14 ^c
	3	55.15±0.35 ^e	66.20±0.00 ^d	77.35±0.35	2.76±0.11 ^d
Two-way ANOVA- <i>p</i>					
Factor A (level)		0.031	0.209	0.026	<0.001
Factor B (time)		<0.001	0.009	0.557	<0.001
Factor A × Factor B		0.493	0.002	0.066	0.043

Mean values ± standard deviation. The mean values with identical letters in specific columns are not significantly different at a 0.05 level of confidence. T_O – onset; T_P – peak, T_E – end temperatures, ΔH – transition enthalpy

Both the concentration of kale and the storage time significantly influenced the values of the T_O, as indicated by the statistical analysis. In the case of T_P value, the statistical variation was smaller, because the concentration of the kale powder addition had no significant effect, and the trend of increasing values for this parameter was observed solely for the sample with the highest concentration of lyophilisate. On the contrary, in the case of the T_E, the concentration of the kale lyophilisate content had a significant effect ($p >$

0.05), while the storage time did not ($p < 0.05$). As in the case of the T_P value, a clear trend associated with the increase in the T_E was observed in the case of the largest 9% concentration of the kale powder in the recipe. A significant effect ($p < 0.05$) of the level of addition, storage time, and their combination was found on the enthalpy of transition value, which corresponds to the energy required for the disintegration of retrograded amylopectin. In each case, the enthalpy values increased with the storage period, indicating the

progressive phenomenon of amylopectin retrogradation. There was no significant effect of the concentration of the kale lyophilisate at 3% or 6% on the value of this parameter. Only the samples with 9% concentration of the kale powder were characterized by a significantly ($p < 0.05$) lower enthalpy value compared to the others.

Similarly, in the following days of storage, significantly ($p < 0.05$) lower enthalpy values were found for 9% concentration of the kale lyophilisate. A high positive correlation was also observed between the enthalpy of decomposition of retrograded amylopectin and crumb hardness ($r = 0.8698$) and chewiness ($r = 0.7875$).

CONCLUSIONS

Considering the obtained results, it was found that despite the optimisation of the water content in the recipe, the kale lyophilisate introduced into it weakened the dough structure, which is confirmed by the increase in the value of the tangent of the phase shift angle and instant compliance, as well as the decrease in zero shear viscosity, cohesion, and consistency in relation to the control dough. The addition of the kale powder to the recipe reduced the volume of the loaves obtained and contributed to a decrease in crumb porosity and pore density, as well as an increase in the count of large pores. The lowest content of the kale lyophilisate had a

positive effect on the acceptability of the taste, while the higher content 6% and 9% decreased the acceptability of all organoleptic features of the bread, except for the taste. Powdered kale added to bread reduced the rate of crumb hardening and, at the highest concentration, increased the enthalpy of decomposition of retrograded amylopectin. The results obtained in these studies indicate that the kale lyophilisate can be a valuable ingredient of gluten-free bread, increasing its nutritional value and reducing the staling process, but due to its sensory acceptability, its concentration in the recipe should be optimized.

REFERENCES

1. Agarwal, A., Raj, N., & Chaturvedi, N. (2017). A comparative study on proximate and antioxidant activity of *Brassica oleracea* (Kale) and *Spinacea oleracea* (Spinach) leaves. *International Journal of Advanced Research in Biological Sciences (IJARBS)*, 4(4), 22–29. <https://doi.org/10.22192/ijarbs.2017.04.04.004>
2. Becerra-Moreno, A., Alanís-Garza, P. A., Mora-Nieves, J. L., Mora-Mora, J. P., & Jacobo-Velázquez, D. A. (2014). Kale: an excellent source of vitamin C, pro-vitamin A, lutein and glucosinolates. *CyTA - Journal of Food*, 12(3), 298–303. <https://doi.org/10.1080/19476337.2013.850743>
3. Bhupathiraju, S. N., Wedick, N. M., Pan, A., Manson, J. E., Rexrode, K. M., Willett, W. C., Rimm, E. B., & Hu, F. B. (2013). Quantity and variety in fruit and vegetable intake and risk of coronary heart disease. *American Journal of Clinical Nutrition*, 98(6), 1514–1523. <https://doi.org/10.3945/ajcn.113.066381>
4. Carter, P., Gray, L. J., Troughton, J., Khunti, K., & Davies, M. J. (2010). Fruit and vegetable intake and incidence of type 2 diabetes mellitus: Systematic review and meta-analysis. *BMJ (Clinical Research Ed.)*, 341, c4229. <https://doi.org/10.1136/bmj.c4229>
5. Carvalho, J. C. S., Romoff, P., & Da Lannes, S. C. S. (2018). Improvement of nutritional and physicochemical proprieties of milk chocolates enriched with kale (*Brassica oleracea* var. *acephala*) and grape (*Vitis vinífera*). *Food Science and Technology*, 38(3), 551–560. <https://doi.org/10.1590/fst.15018>
6. Di Noia, J. (2014). Defining powerhouse fruits and vegetables: a nutrient density approach. *Preventing Chronic Disease*, 11, 130390. <https://doi.org/10.5888/pcd11.130390>
7. Drabińska, N., Ciska, E., Szmatowicz, B., & Krupa-Kozak, U. (2018). Broccoli by-products improve the nutraceutical potential of gluten-free mini sponge cakes. *Food Chemistry*, 267, 170–177. <https://doi.org/10.1016/j.foodchem.2017.08.119>
8. Emebu, P. K., & Anyika, J. U. (2010). Vitamin and antinutrient composition of kale (*Brassica oleracea*) grown in Delta State, Nigeria. *Pakistan Journal of Nutrition*, 10(1), 76–79. <https://doi.org/10.3923/pjn.2011.76.79>
9. Kahlon, T. S., Chapman, M. H., & Smith, G. E. (2007). In vitro binding of bile acids by spinach, kale, brussels sprouts, broccoli, mustard greens, green bell pepper, cabbage and collards. *Food Chemistry*, 100(4), 1531–1536. <https://doi.org/10.1016/j.foodchem.2005.12.020>
10. Kasprzak, K., Oniszcuk, T., Wójtowicz, A., Waksmundzka-Hajnos, M., Olech, M., Nowak, R., Polak, R., & Oniszcuk, A. (2018). Phenolic acid content and antioxidant properties of extruded corn

snacks enriched with kale. *Journal of Analytical Methods in Chemistry*, 2018, 7830546. <https://doi.org/10.1155/2018/7830546>

11. Kim, S. Y., Yoon, S., Kwon, S. M., Park, K. S., & Lee-Kim, Y. C. (2008). Kale juice improves coronary artery disease risk factors in hypercholesterolemic men. *Biomedical and Environmental Sciences*, 21(2), 91–97. [https://doi.org/10.1016/S0895-3988\(08\)60012-4](https://doi.org/10.1016/S0895-3988(08)60012-4)
12. Klopsch, R., Baldermann, S., Hanschen, F. S., Voss, A., Rohn, S., Schreiner, M., & Neugart, S. (2019). *Brassica*-enriched wheat bread: Unraveling the impact of ontogeny and breadmaking on bioactive secondary plant metabolites of pak choi and kale. *Food Chemistry*, 295, 412–422. <https://doi.org/10.1016/j.foodchem.2019.05.113>
13. Korus, A. (2020). Changes in the content of minerals, B-group vitamins and tocopherols in processed kale leaves. *Journal of Food Composition and Analysis*, 89, 103464. <https://doi.org/10.1016/j.jfca.2020.103464>
14. Korus, A., Witczak, M., Korus, J., & Juszczak, L. (2023). Dough rheological properties and characteristics of wheat bread with the addition of lyophilized kale (*Brassica oleracea* L. var. *sabellica*) powder. *Applied Sciences*, 13(1), 29. <https://doi.org/10.3390/app13010029>
15. Korus, J., Ziobro, R., Witczak, T., Kapusniak Jochym, K., & Juszczak, L. (2021). Effect of Ooteryl succinic anhydride (OSA) modified starches on the rheological properties of dough and characteristic of the gluten-free bread. *Molecules*, 26(8). <https://doi.org/10.3390/molecules26082197>
16. Krupa-Kozak, U., Drabińska, N., Bączek, N., Šimková, K., Starowicz, M., & Jeliński, T. (2021). Application of broccoli leaf powder in gluten-free bread: An innovative approach to improve its bioactive potential and technological quality. *Foods*, 10(4). <https://doi.org/10.3390/foods10040819>
17. Kural, B. V., Küçük, N., Yücesan, F. B., & Örem, A. (2011). Effects of kale (*Brassica oleracea* L. var. *acephala* DC) leaves extracts on the susceptibility of very low and low density lipoproteins to oxidation. *Indian Journal of Biochemistry & Biophysics*, 48(5), 361–364.
18. Majzoobi, M., Poor, Z. V., Jamalain, J., & Farahnaky, A. (2016). Improvement of the quality of gluten-free sponge cake using different levels and particle sizes of carrot pomace powder. *International Journal of Food Science & Technology*, 51(6), 1369–1377. <https://doi.org/10.1111/ijfs.13104>
19. Ranawana, V., Campbell, F., Bestwick, C., Nicol, P., Milne, L., Duthie, G., & Raikos, V. (2016). Breads fortified with freeze-dried vegetables: Quality and nutritional attributes. Part II: Breads not containing oil as an ingredient. *Foods*, 5(3). <https://doi.org/10.3390/foods5030062>
20. Ren, Y., Linter, B. R., Linforth, R., & Foster, T. J. (2020). A comprehensive investigation of gluten free bread dough rheology, proving and baking performance and bread qualities by response surface design and principal component analysis. *Food & Function*, 11(6), 5333–5345. <https://doi.org/10.1039/d0fo00115e>
21. Ribotta, P. D., & Le Bail, A. (2007). Thermo-physical assessment of bread during staling. *LWT - Food Science and Technology*, 40(5), 879–884. <https://doi.org/10.1016/j.lwt.2006.03.023>
22. Royston, K. J., & Tollefsbol, T. O. (2015). The Epigenetic Impact of Cruciferous Vegetables on Cancer Prevention. *Current Pharmacology Reports*, 1(1), 46–51. <https://doi.org/10.1007/s40495-014-0003-9>
23. Šamec, D., Urlić, B., & Salopek-Sondi, B. (2019). Kale (*Brassica oleracea* var. *Acephala*) as a superfood: Review of the scientific evidence behind the statement. *Critical Reviews in Food Science and Nutrition*, 59(15), 2411–2422. <https://doi.org/10.1080/10408398.2018.1454400>
24. U.S. Department of Health and Human Services and U.S. Department of Agriculture. (2015). 2015 – 2020 Dietary Guidelines for Americans. 8th Edition. December 2015. <https://health.gov/our-work/food-nutrition/previous-dietary-guidelines/2015>
25. Wang, N., Xu, Y., Chao, H., Zhang, M., Zhou, Y., & Wang, M. (2020). Effects of celery powder on wheat dough properties and textural, antioxidant and starch digestibility properties of bread. *Journal of Food Science and Technology*, 57(5), 1710–1718. <https://doi.org/10.1007/s13197-019-04204-8>
26. Witczak, M., Korus, J., Ziobro, R., & Juszczak, L. (2019). Waxy starch as dough component and anti-staling agent in gluten-free bread. *LWT - Food Science and Technology*, 99, 476–482. <https://doi.org/10.1016/j.lwt.2018.10.009>
27. Witczak, T., Juszczak, L., Ziobro, R., & Korus, J. (2017). Rheology of gluten-free dough and physical characteristics of bread with potato protein. *Journal of Food Process Engineering*, 40(3), e12491. <https://doi.org/10.1111/jfpe.12491>
28. World Health Organization. (2020). *Healthy diet*. <https://www.who.int/news-room/fact-sheets/detail/healthy-diet>