Whole wheat bread enriched with silver fir (Abies alba Mill.) needles extract: technological and antioxidant properties

Journal of the
Science of Food and
Agriculture

SCI
where science
meets business

Whole wheat bread enriched with silver fir needles (Abies alba Mill.) extract: technological and antioxidant properties

Journal:	*Journal of the Science of Food and Agriculture*
Manuscript ID	JSFA-21-1453.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	29-Nov-2021
Complete List of Authors:	Parenti, Ottavia; National Research Council, Institute for Bioeconomy Albanese, Lorenzo; National Research Council, Institute for Bioeconomy Guerrini, Lorenzo; University of Florence, DAGRI - Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali Zanoni, Bruno; University of Florence, DAGRI, Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali Zabini, Federica; National Research Council, Institute for Bioeconomy MENEGUZZO, FRANCESCO; National Research Council, Institute for Bioeconomy
Key Words:	Hydrodynamic cavitation, bioeconomy, added-value bread, green extraction, antioxidant capacity, functional bread

Title

~~The use of aqueous extract from silver fir needles (*Abies Alba* Mill.) as enrichment material for unrefined~~ Whole wheat bread enriched with silver fir needles (*Abies alba* Mill.) extract: technological and antioxidant properties

Running title

Bread enriched with silver fir needles extract: technological and antioxidant properties

Authors

Ottavia Parenti[1], Lorenzo Albanese[1], Lorenzo Guerrini[2]*, Bruno Zanoni[23], Federica Zabini[1], Francesco Meneguzzo[1]

1 Institute for Bioeconomy, National Research Council, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Florence, Italy

2 ~~Department of Agricultural, Food and Forestry Systems Management (DAGRI), University of Florence, Piazzale Delle Cascine 16, 50144, Florence, Italy~~ Department of Land, Environment, Agriculture and Forestry (TeSAF), University of Padova, Viale dell'Università,16, 35020 Legnaro (PD), Italy

3 Department of Agricultural, Food and Forestry Systems Management (DAGRI), University of Florence, Piazzale Delle Cascine 16, 50144, Florence, Italy

* Corresponding author. E-mail address: lorenzo.guerrini@unifi.it, ~~Department of Agricultural, Food and Forestry Systems Management (DAGRI), University of Florence, Piazzale Delle Cascine 16,~~

~~50144, Florence, Italy~~ Department of Land, Environment, Agriculture and Forestry (TeSAF), University of Padova, Viale dell'Università,16, 35020 Legnaro (PD), Italy

Abstract

BACKGROUND: The interest of consumers, market and scientific research for added-value foods obtained with environmentally sustainable productive-chains is increasing. Silver fir needles (*Abies alba* Mill.) (SFN), often by-products of forest management and logging, represent an unexploited source of bioactive compounds.

RESULTS: For the first time, SFN (*Abies* ~~*A*~~*alba* Mill.) aqueous extract obtained through controlled hydrodynamic cavitation (HC) was used to enrich ~~unrefined~~ whole wheat flour bread. The first trial found that 35% SFN extract addition was the absolute threshold of taste perception. The second trial investigated dough rheological properties, and bread technological and antioxidant properties in samples enriched with 35% and 100% SFN extract compared to the control (0% SFN extract). SFN extract significantly increased bread antioxidant capacity in both 35% and 100% SFN fresh ~~enriched~~ breads of approx. 42.5% and 87%, respectively, and in 100% SFN bread samples after 72 h of storage of approx. 76%. Enrichment of 35% showed higher alveograph dough extensibility (approx. 11%), and different bread texture in terms of hardness, springiness, and chewiness. Enrichment of 100% SFN extract significantly ~~exhibite~~ improved dough and bread technological quality: it increased alveograph dough extensibility – *L* (approx. 18%), swelling index – *G* (approx. 8%), flour strength – *W* (approx. 14%), and showed the highest increase in bread specific volume (approx. 0.200 L/kg).

CONCLUSIONS: SFN aqueous extract produced with controlled HC appeared ~~as~~ a valuable technical material for the manufacturing of added-value and functional breads. ~~The obtained results encourage further research on SFN chemical composition, the molecular effects accounting for the~~

~~observed macroscopic results, the bio-accessibility and bioavailability of antioxidant compounds and the sensory evaluation of enriched breads~~.

Keywords

Hydrodynamic cavitation; bioeconomy; added-value bread; functional bread; antioxidant capacity; green extraction

1. Introduction

The interest of consumer, market and scientific research for high nutritional value foods obtained by environmentally sustainable productive-chains is constantly increasing. Vegetable by-products from underutilized forestry resources and agri-food wastes can be used as sources of bioactive compounds to produce added-value and functional foods.[1–4] Wheat bread is a staple food consumed worldwide, hence it has been frequently considered for enrichment strategies, as it can be a good carrier of bioactive compounds.[4–6] Indeed, such components, in particular antioxidant compounds, have shown to play a crucial role in the prevention of non-communicable diseases such as cardiovascular diseases, type 2 diabetes, some kind of cancers and neurodegenerative diseases.[7] Different by-products have been studied as bread enrichment ingredients, such as green parts of buckwheat plant,[8] coriander leaves powder,[9] quinoa leaves powder,[10] lettuce waste flours,[11] artichoke stem powder,[12] industrial broccoli co-products,[13] dried olive leaves,[14] flour from olive stones,[15] and stinging nettle leaves.[16] In all the above studies, by-products were added in dried forms, produced using specific treatments that can be cost-intensive, and energy-, resource- and time-consuming. As well, Meneguzzo et al.[17] showed that when adding dried ground materials to bread, most of antioxidant compounds, such as polyphenols, remained bound to cell walls materials and hence they result substantially unavailable for human absorption. By contrast, scant scientific research has been performed on extracts of by-products in the form of aqueous solutions or purée for bread production.[18–22]

Within vegetable sources, extracts obtained from needles, bark, and wood of coniferous tree species, have been recently studied for their chemical composition, biological and pharmacological activities as well as for their application in the fields of nutrition, health, and medicine.[23] Furthermore, coniferous sprouts and needles have been used in contemporary cuisine to produce sorbets, ice-creams, and cocktails.[24,25]

The increased attention to environmental sustainability has led to introduce innovative green extraction techniques to recover bioactive compounds from by-products.[26] Controlled hydrodynamic cavitation (HC) is emerging as a promising green and innovative technique for the extraction of valuable components from forestry by-products and agri-food wastes.[27–31] The principle of HC is based on the passage of a liquid through one or more constrictions (such as Venturi tubes and orifice plates), resulting in the generation, growth and collapse of microbubbles due to pressure variations in the liquid flow. The violent collapse of the cavitation bubbles results in the generation of localized hot spots where physical and chemical changes to the target matrix occur.[32–34] Albanese et al. (2019)[30], applying a HC-based extraction process in water only to a forest by-product, i.e., silver fir needles (SFN) (*Abies Aalba* Mill.), obtained aqueous extracts with high antioxidant capacity. The highest antioxidant level of the SFN extract was comparable to the levels reported for some reference antioxidant molecules.[30]

To the best of the authors' knowledge, in the current literature the use of aqueous extract of *Abies Aalba* Mill. SFN obtained by means of controlled HC to produce wheat bread has not been investigated. Therefore, the aim of the present study was to test the effect of SFN aqueous extract on the rheology of ~~unrefined~~ whole wheat (i.e., WWF) dough, and on the technological and antioxidant properties of ~~unrefined~~ WWF wheat bread.

2. Materials and Methods

2.1 Materials

The experimental trials were performed with one batch of a sp. *Triticum aestivum* L., cv. Bologna. The wheat was grown in Montespertoli (Florence, Italy) during the 2020-2021 growing season. ~~Whole wheat flour~~ WWF, i.e., extraction rate ~~98 g/100 g~~ 980 g/kg dry kernel, ash content ~~1.3-1.7 g/100 g~~ 13-17 g/kg dm~~[32]~~ [35] was obtained using a stone grinding mill and a sieve (two consecutive passages through a 1,100–1,200 µm sieve) at the Molino Paciscopi (Montespertoli, Florence, Italy). Fresh brewer's yeast (Original, Casteggio, Italy) was purchased at a local market (Florence, Italy). All chemical reagents were of analytical grade. 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Sigma-Aldrich (St. Louis, MO, USA), methanol from VWR chemicals (BDH Prolabo, Leuven, Belgium), and DPPH (2,2-diphenyl-1- picrylhy- drazyl) from Tokyo Chemical Industry (TCI) (Tokyo, Japan).

2.2 The experimental design

Two different experimental trials were carried out to test the effect of SFN extract:

(i) A first trial (*T1*) to test different levels of SFN extract addition in ~~unrefined~~ WWF bread to determine the absolute threshold of taste perception;

(ii) A second trial (*T2*) for an in-depth evaluation of the technological quality of three selected levels of SFN extract supplementation on dough, fresh breads and on bread during storage time.

2.2.1 Preliminary trials

Two preliminary trials were performed: (i) An optimization trial (*OT*) and (ii) An exploratory trial (*ET*) as follows:

(i) The optimization trial (*OT*) was performed using the Design of Experiment (DoE) Methodology following the approach adopted in a previous study.[30] The kneading conditions tested were (i) Kneading time – *T* (i.e., 8, 11, 14, 17, 20 min), and Water amount – *W* (i.e., ~~55.5, 59.5, 63.5, 67.5, 71.5%~~ 555, 595, 635, 675, 715 g/kg of flour); bread quality was evaluated in terms of bread specific volume. The above levels of water amount and kneading time were selected based on preliminary trials. The application of a Central Composite Circumscribed (CCC) Design based on RSM allowed to estimate bread specific volume within the selected range of the experimental variables. The kneading conditions (*T*, *W*) that maximized bread specific volume (i.e., *T* = 13.5 min and *W* = ~~71.5% (w/flour w)~~ 715 g/kg of flour were adopted to properly test the effect of SFN extract supplementation in the experimental trials *T1*, and *T2*.

(ii) The exploratory trial (*ET*) tested the substitution of the water required in the bread recipe with different levels of SFN extract (i.e., 0, 20, 40, 60, 80, and 100% w/water w) to approximately identify the levels that were closer to the absolute threshold of taste perception. Bread samples were evaluated by panellists familiar with cereal products, and the results were used to define the levels of SFN supplementations to be tested in the experimental trial 1 (*T1*).

2.2.2 Experimental trial 1 (T1)

Basing on the *ET* results, in the experimental trial 1 (*T1*) the substitution of water with SFN extract was tested at the following levels: 0% (*SFN0-T1*), 25% (*SFN25-T1*), 35% (*SFN35-T1*), 45% (*SFN45-T1*), 55% (*SFN55-T1*), 100% (*SFN100-T1*) (w/water w) to determine the level that corresponds to the absolute threshold of taste perception. Furthermore, bread specific volume, and crumb and crust moisture were evaluated.

2.2.3 Experimental trial 2 (T2)

The experimental trial 2 (*T2*) tested the effect of the supplementation of ~~unrefined~~ WWF bread with the following levels of SFN extract:

- SFN extract = 0% w/water w, i.e. the control bread – *SFN0-T2*;
- SFN extract = 35% w/water w, i.e. the bread enriched with the SFN extract level that, according to *T1* results (see Section 3.1), corresponded to the absolute threshold of taste perception – *SFN35-T2*; with this substitution level we aimed to test the effect obtainable with the maximum enrichment amount of SFN extract that did not alter the bread sensory profile;
- SFN extract = 100% w/water w, i.e. the sample in which the total water content of bread was substituted with SFN extract – *SFN100-T2*; with this substitution level we aimed to test the maximum obtainable effect of bread enrichment with SFN extract. In this sample the addition of SFN extract impacted both the nutritional and sensory profile (see Section 3.2), resulting in a clearly different bread type that could be included in the special bread category.~~[34,35]~~ [36,37]

The evaluation of the ~~unrefined~~ WWF dough and bread enriched with SFN extract included: rheological properties (Alveographic test), bread specific volume, crumb and crust moisture, Texture Profile Analysis (TPA), bread antioxidant capacity (DPPH). The effect of storage time (i.e., 72

h) was also tested on crumb and crust moisture, Texture Profile Analysis (TPA), and bread antioxidant capacity (DPPH assay).

2.3 Silver fir needles samples and Hydrodynamic Cavitation (HC) extraction

Silver fir needles (*Abies ~~A~~alba* Mill.) were manually collected the first days of February 2021 in a mixed silver fir forest in the northern Tuscany, Italy, near the town of San Marcello – Piteglio (Pistoia, Italy) at an altitude of about 1,050 m a.s.l. on the southern slopes of the Apennines mountain range, characterized by a mid-mountain temperate climate with abundant precipitation.

The day after harvesting, needles were manually removed from the twigs until a batch of 2.170 kg (fresh weight) was obtained. While nothing would prevent using a higher amount of SFN, their manual removal from the twigs was quite time consuming and we chose this method to prevent any damage to SFN. The extraction was performed using controlled Hydrodynamic Cavitation (HC) technique according to Albanese et al. (2019)[30], which involved a closed hydraulic circuit comprising a fixed cavitation reactor in the form of a Venturi tube and a main tank. An amount of 130 L tap water was used as the only solvent. SFN mass to water ratio corresponded to approx. ~~1.67% (w/w fresh weight)~~ 16.7 g/kg for fresh SFN and to approx. ~~to 0.90% (w/w dry weight)~~ 9.0 g/kg for dry SFN, since the moisture of fresh silver fir needles, measured by gravimetry at 105°C until constant weights were reached, was ~~46.32 ± 0.94 %~~ 463.2 ± 9.4 g/kg. The process was performed at atmospheric pressure, without any control on the temperature of the circulating mixture; temperature, power and consumed electricity were monitored during the HC extraction. The whole needles were mixed with water and ice, then ground with an immersion blender to reduce their average length before performing the extraction. The HC extraction was performed until the temperature rise, resulting from the balance between the mechanical energy supplied by the pump impeller and the heat loss through the uninsulated walls of the hydraulic circuit, reached 47°C. The total extraction time was 60 min. The highest temperature value was selected to obtain the highest antioxidant capacity in the SFN extract according to the results reported by Albanese et al. (2019)[30]. No technical problem arose during the extraction process. Figure 1 shows the evolution of the temperature and the Cavitation Number during the process. The temperature increased from 14.5°C to 47°C, while the Cavitation Number was in the range 0.30 to 0.35 until 48 minutes of process time, later decreasing to 0.25 when the absorbed power increased from an average of 5,900 W to 6,200 W, likely due to the partial hydrolysis of cellulose and hemicellulose, and/or to the further size reduction of the SFN particles.

While a detailed description of the Cavitation Number, its meaning and evaluation method is available in a previous work,[29] its levels during the process suggest a fully developed, moderate to intense cavitation. The consumed electricity during the process was 6.25 kWh, corresponding to a specific energy consumption of 2.88 kWh per kg of fresh SFN. After the HC-based extraction, the SFN aqueous extract was filtered with a steel sieve and stored in 20 L tanks at room temperature (22 ± 2°C) until use.

2.4 Bread-making

500 g dough batches were prepared, and the straight dough method was applied. The standard bread formula included: flour (310 g), fresh brewer's yeast (13 g), and the amount of water obtained in the Optimization Trial (*OT*). Kneading was performed with Kitchen Aid Professional Mixer (5KSM185PS, KitchenAid, St. Joseph, Michigan, USA) equipped with a dough hook (model KSM35CDH) at room conditions (T = 22 ± 2 °C, relative humidity = 50%). Then, dough samples were placed in a bread-making machine (Pain doré, Moulinex, Ecully, France) to perform the leavening phase for 90 min at room conditions (T = 22 ± 2°C, relative humidity = 50%), and they were baked at 150 °C for 50 min. Three bread-making replicates were performed in *T1* and *T2*. Bread samples

were characterised by round shape, weight-size of approx. 0.4 kg, specific volume in the range of 3.0-3.5 L/kg, fine-grained crumb, and thick and crispy hazel crust.

After baking, bread samples were cooled to room temperature (T = 22 ± 2°C) at room moisture (relative humidity = 50%) prior to bread quality evaluation. Fresh samples were measured after 24 h from the bread-making process. Stored bread samples were put in paper bags at room conditions (T = 22 ± 2°C, relative humidity = 50%), and quality evaluation was performed 72 h after the bread-making process.

2.5 Measurements methods

2.5.1 Dough rheology

The Alveograph test was performed according to the standard method (AACC 54–30.02), except for the dough moisture content; indeed, the same water (i.e., the amount determined in the *OT*) and SFN extract amounts (i.e., 0, 35, 100%, w/water w) used for the bread-making trial were added. The measured ~~A~~alveographi~~c~~ parameters were the following: dough tenacity – *P* (mm H_2O), defined as the maximum overpressure; dough extensibility – *L* (mm), the average bubble length at rupture; swelling index – *G* (mm), the square root of the volume of air necessary to inflate the dough bubble until it ruptures; flour strength – *W* (10^{-4} J), the energy required to inflate the dough bubble to the point of rupture; and tenacity-extensibility ratio – *P/L*.~~[36]~~ [38] Three treatment replicates were performed.

2.5.2 Bread quality

The bread technological quality was evaluated considering the following parameters: bread volume (L), bread specific volume (L/kg), crumb and crust moisture contents (~~%, g/100 g~~ g/kg), and Texture

Profile Analysis (TPA) ~~(hardness – N, cohesiveness, chewiness – N*mm, springiness – mm)~~ according to Parenti et al. (2020a)~~[37]~~ [39]. Bread volume (L) was measured using the standard millet displacement method (AACC, 10-05.01), specific volume (L/kg) was determined as the ratio between total volume and mass, and crumb and crust moisture (g/kg) were measured by gravimetry at 105 °C until constant weights were reached. TPA was carried out by two-bite compression using a Texture Analyzer (Stable Micro Systems, UK), equipped with a circular flat-plate probe (diameter: 30 mm), and hardness (N), cohesiveness, chewiness (N*mm) and springiness (mm) were measured on three slices (1.5 cm thickness) of each bread sample. Three measure replicates and three treatment replicates were performed for the above parameters.

2.5.3 Antioxidant Capacity (DPPH assay)

Fresh bread slices were put in plastic bags and stored at -21°C before performing the extraction of antioxidant compounds. Bread slices were thawed at room temperature the night before the DPPH assay to have fresh bread slices the following day. Samples of fresh bread crumb were finely ground using a mixer and 5.00 g of ground crumb were extracted with 30 mL of a solution methanol:water (80:20, v/v) for 1 h at room temperature under constant stirring. Then, samples were centrifuged for 10 min at ~~5,000 rpm~~ 2,370 x g at 4°C. Antioxidant capacity (AC) was determined on the supernatants stored in dark at 4°C the day after the extraction.

DPPH* scavenging activity was determined following the modified method of Brand-Williams et al. (1995)~~[38]~~ [40] as described by Zielinski et al. (2008)~~[39]~~ [41]. In the DPPH assay, antioxidant compounds present in the sample reduce the free radical 2,2-diphenyl-1-picrylhydrazyl, which has an absorption maximum at 517 nm. DPPH reagent standard solution was prepared at a concentration of 0.8 g/L in methanol 100%; working solution of DPPH* were prepared fresh daily by diluting the stock solution with methanol 100%. The absorbance of the sample prepared with 250 µL of the DPPH* working solution and 2.1 mL of 80% methanol (v/v) was measured as the absorbance of the free radical, and the sample prepared with 250 µL of 100% methanol, and 2.1 mL of 80% methanol (v/v) was used as blank. Test samples were prepared in disposable cuvettes by adding 100 µL of bread crumb extracts to 2 mL of 80% methanol (v/v) and 250 µL of the DPPH* working solution. The absorbance at 517 nm was measured with a spectrophotometer after 30 min of incubation in the dark at room temperature (22 ± 2°C). The Trolox standard solutions (concentrations 0.1-2 mM) in 80% methanol (v/v) were assayed in the same conditions, and then the DPPH* scavenging activity of the samples was expressed in terms of milligrams of Trolox equivalent AC per gram of dry bread crumb (mg TEAC/g dm).

2.5.4 Absolute threshold of taste perception – Method of Limits

The sensitivity of a given sensory system to the relevant stimuli can be expressed as an absolute threshold. Absolute threshold refers to the minimum amount of stimulus energy that must be present for the stimulus to be detected 50% of the time. The absolute threshold of taste perception of SFN extract in unrefined WWF bread was determined according to the “Method-of-Limits”.[40][42] Fresh samples of unrefined WWF breads containing different amounts of SFN extract (0%, 25%, 35%, 45%, 55%, and 100% w/water w) were given in a random order to a panel of twenty panellists expert in food and beverage tasting and familiar with cereal products. The number of judges was chosen according to Harris (1960)[41][43] and Gonázlez et al. (1998)[42][44]. The sensory test was performed in compliance with the principles laid down in the Declaration of Helsinki, and all subjects provided informed, written consent prior to participation in agreement with the Italian ethical requirements on research activities and personal data protection (Law Decree 30.6.03, 196).[45]
The panel was asked to taste and swallow the bread samples, and then to answer for each sample if they perceived or not the presence of SFN extract. Water was provided to cleanse the palate

between the bread samples. The panel was instructed that the breads were prepared with different amounts of SFN extracts. According to the Method of Limits, the SFN bread sample that was perceived by the 50% of the panel was considered the absolute threshold of taste perception.

2.6 Data processing

To evaluate the effect of the different levels of SFN addition on bread technological quality, *T1* experimental data were analysed using regression models. The best data fitting was obtained using the simple linear model for the independent variable SFN extract according to the following equation:

$$y_{obs} = b_0 + b_1\ SFN + error \qquad [1]$$

where b_0 is a constant (the intercept) and b_1 represents the main effect of the SFN extract addition. A one-way ANOVA was used to assess the significance of the independent variable, i.e. SFN extract, on bread technological parameters.

Method of limits data were treated according to the indication of Meilgaard et al. (2006)[40 42]. Briefly, the frequencies of perception were calculated and the SFN addition corresponding to the frequency at the level of 0.5 was chosen as the threshold.

A one-way ANOVA was performed in *T2* to test significant differences on the technological and antioxidant properties of fresh bread samples due to the addition of the SFN extract. A two-way ANOVA was used to assess significant differences on bread technological and antioxidant properties due to SFN extract addition, storage time and their interaction (SFN extract*storage time). The Tukey's honestly significant difference (HSD) test was used as post-hoc test. The data were analysed with R software.

3. Results and Discussion

3.1 Experimental Trial 1 (T1)

The addition of SFN extract had a significant linear relationship with the bread specific volume ($p < 0.01$), whereas not significant linear relationships ($p > 0.05$) with crumb and crust moistures were observed.

Bread specific volume significantly increased ($p < 0.01$) with the amount of SFN extract addition, starting from a value of 3.302 ± 0.017 L/kg in *SFN0-T1*, and linearly increasing up to a value of 3.497 ± 0.065 L/kg in *SFN100-T1*. Crumb and crust moistures were not significantly affected by SFN extract, showing average values of ~~47.52 ± 0.43%~~ 475.2 ± 4.3 and ~~24.34 ± 0.76%~~ 243.4 ± 7.6 g/kg, respectively.

In the literature, the addition of by-products from different sources, despite determining the pursued increase of bread AC, showed contradictory effects on dough properties, i.e. the increase/decrease of dough mixing time and dough strength;[2,4,5]; furthermore, the impact on bread technological quality was often detrimental.[6] It could be hypothesised that the amount, chemical form and typology of SFN antioxidant compounds make them follow a similar fate as ascorbic acid, one of the most common flour treatment agents. Indeed, ascorbic acid is an antioxidant compound but in the presence of oxygen gas and an enzyme (ascorbic acid oxidase) it is converted in the dehydro form which has the potential to take part in oxidation reactions such as the SH/SS inter-change and to help stabilize the gluten protein network.[46] Similar reactions may occur when the dough system is enriched with SFN antioxidant agents, accounting for the positive effect observed on bread specific volume. However, further research is required to gain a deeper insight into the chemical-physical phenomena occurring in SFN enriched dough.

The addition of SFN extract did not impact the colour of bread crumb and crust evaluated on visual assessment. The level of SFN extract supplementation perceived by the 50% of the panellists and hence representing the absolute threshold of taste perception was 35% (i.e., *SFN35-T1* sample). This means that 35% was the maximum enrichment level of SFN that did not produce perceivable changes on the bread taste. The percentages of positive answers related to the taste perception of SFN extract in the other bread samples were the following: 0% *SFN0-T1*, 21% *SFN25-T1*, 79% *SFN45-T1* and 93% both *SFN55-T1* and *SFN100-T1*.

3.2 Experimental trial 2 (T2)

In *T2* the rheological properties of ~~unrefined~~ WWF dough and the technological and antioxidant properties of ~~unrefined~~ WWF breads supplemented with three selected levels of SFN extract, i.e. 0% (*SFN0-T2*) – the control sample, 35% (*SFN35-T2*) – the sample corresponding to the absolute threshold of SFN extract perception, and 100% (*SFN100-T2*) – the sample with the highest amount of SFN extract, were evaluated.

3.2.1 Dough rheology – Alveograph test

Data about dough rheology are reported in Table 1 and the most relevant results are shown in Figure 2. SFN extract had a significant effect on dough extensibility – *L* ($p < 0.01$), dough swelling index – *G* ($p < 0.05$) and flour strength – *W* ($p < 0.001$) (Figure 2), whereas no significant differences ($p > 0.05$) were found for dough tenacity – *P*, tenacity to extensibility ratio – *P/L*, and elasticity index – *Ie*. SFN extract significantly enhanced the dough extensibility – *L*, regardless of the level of SFN extract supplementation. Indeed, both *SFN35-T2* and *SFN100-T2* dough samples were characterised by a significantly higher *L* value than that of the control sample, showing an increase of approx. 11% and 18%, respectively (Figure 2).

Conversely, dough swelling index – *G* was significantly increased by SFN extract only in *SFN100-T2* dough sample (approx. 8%), whereas *SFN35-T2* dough sample showed a slight and not significant growth of the parameter (approx. 3%) (Figure 2).

The same result was observed for the flour strength – *W*, which showed a significant increase of approx. 14% in *SFN100-T2* dough sample and a not significant increase of approx. 2.5% in *SFN35-T2* dough sample compared to the control sample (*SFN0-T2*) (Figure 2).

In the literature, the enrichment of dough with antioxidant compounds from different by-products has shown inconsistent results on dough rheological properties.[6] The hypothesis could be advanced that the observed effects were related to the amount, nature and physical form of by-product used as well as to the type and concentrations of antioxidant compounds.

Several studies reported that antioxidant compounds caused a weakening of the dough structure, whereas some others reported positive effects of the incorporation of phenolic compounds on the dough strength.~~[43–45]~~ [47–49] Our results were consistent with these latter studies since both 35% and 100% substitution levels of bread dough water with SFN extract significantly improved the dough extensibility – *L*. Furthermore, *SFN100-T2* also showed an increase of the flour strength – *W*, and the dough swelling index – *G*, which are positive results for ~~unrefined wheat~~ WWF dough generally characterised by poor rheological properties.~~[46]~~ [50] Several interactions between antioxidants – proteins, and antioxidants – starch have been investigated and showed variable results as a function of different factors (i.e., type and amount of antioxidant compounds, dough ingredients, water amount, kneading conditions, pH, temperature etc.).~~[47,48]~~ [51,52] Antioxidant compounds can form indigestible complexes with flour proteins and starch, they can change the protein secondary structure,~~[47]~~ [51] as well as they can act as reducing agents, leading to the interchange of the gluten disulphide bounds (S-S) with thiol groups (SH), and to a consequent depolymerization of the gluten network.[6] In this study, the enrichment of bread dough with SFN aqueous extract showed positive results on dough rheology, promoting further investigations.

3.2.2 Bread quality

Data about bread quality are reported in Table 2. The addition of SFN extract significantly impacted bread specific volume ($p < 0.001$), hardness ($p < 0.001$), springiness ($p < 0.05$) and chewiness ($p < 0.05$) parameters of bread crumb texture, and on the AC of bread crumb ($p < 0.05$). Storage time significantly affected crumb ($p < 0.01$) and crust moisture ($p < 0.001$), and hardness ($p < 0.05$), cohesiveness ($p < 0.001$) and chewiness ($p < 0.001$) parameters of crumb texture, and the AC of bread crumb ($p < 0.05$). Furthermore, a significant interaction between SFN extract and storage time was observed on springiness ($p < 0.01$).

Bread specific volume showed that the substitution of 100% of bread water with SFN extract (*SFN100-T2* sample) caused a significant increase of approximately 0.200 L/kg compared to the control sample (*SFN0-T2*). Conversely, *SFN35-T2* showed a not significant increase (approximately 0.075 L/kg) of bread specific volume compared to *SFN0-T2* (Table 2). This result was consistent with *T1* data. Furthermore, as previously discussed (Section 3.1), an increase in bread specific volume as a consequence of the inclusion of by-products rich in antioxidant compounds was rarely reported in the literature,[43] [47] whereas most frequently the opposite effect, namely a reduction of bread technological quality, was observed.[6] Since most of past studies used by-products in a dried form and the effects on bread quality are similar to what reported for the inclusion of antioxidant dietary fibres,[49] [53] it could be hypothesised that the amount, type and physical form of the added by-product play a critical role.

Considering the texture of bread crumb, SFN extract significantly increased the hardness (~~ca.~~ approx. 37%), springiness (~~ca.~~ approx. 21%) and chewiness (~~ca.~~ approx. 41%) of *SFN35-T2* sample, whereas no significant differences were observed between *SFN0-T2* and *SFN100-T2* samples (Table 2). Contradictory effects were reported on bread crumb hardness upon the inclusion of antioxidant compounds, since both a decrease and an increase of bread hardness were observed, probably as a result of the characteristics of the added ingredients (i.e., amount, type, physical form, and the kind and concentration of antioxidant compounds).[6] The fact that the significant effects on bread texture parameters were observed only at 35% of SFN extract inclusion may be tentatively ascribed to the following factors: (i) different interactions between flour and SFN extract constituents might occur at different supplementation levels (i.e., different concentration of antioxidant compounds); (ii) the significant increase in bread specific volume of *SFN100-T2* could have compensated for the changes in bread crumb texture. Further investigation is required to achieve a better comprehension of the observed effects.

Consistently with the literature,~~[50,51]~~ [54,55] storage time significantly increased hardness in stored breads compared to fresh breads, and the significant differences observed as a function of SFN extract addition were preserved during storage (Table 2). Both springiness and chewiness were significantly reduced in stored bread compared to fresh samples (Table 2) as a result of the physical-chemical changes occurring during bread storage including starch transformation, starch-gluten interactions and moisture redistribution.~~[50,51]~~ [54,55] Similar to hardness, the trend of chewiness in stored breads was the same observed in fresh breads. Conversely, a significant interaction between SFN extract and storage time was observed on springiness: the significant differences observed as a function of SFN extract addition in fresh breads were not preserved in stored breads, which were all characterised by not statistically different values (Table 2).

The AC of bread crumb significantly increased with the amount of SFN extract supplementation (Table 2). Compared to the control sample (*SFN0-T2*), a significant AC increase of approximately 42.5% was found since 35% SFN supplementation (*SFN35-T2* sample), and *SFN100-T2* sample showed the highest AC increase of approximately 87%. It was shown that the AC level of enriched-breads mainly derives from the phenolics rather than other compounds.[~~47~~ 51] Our results were consistent with the literature, since it was reported that the enrichment of bread with antioxidant compounds often resulted in an increase of bread AC, generally increasing with the amount of by-product: the higher the by-product amount and its AC level, the higher the resulting bread AC level.[2,4,5] Bread enrichment with by-products in a dried form often resulted in a greater AC increase compared to our results, since the use of ingredients in that physical form allowed to introduce high amounts of antioxidant compounds into the bread.[~~52–56~~ 54–60] However, it has been often reported a general impairment of gluten network development as well as bread volume and texture parameters; these effects may be ascribed to the type, amount and physical form of by-product used.[6,~~49~~ 53,~~53~~,57, ~~55~~, 59] Conversely, we observed a significant improvement of bread technological quality coupled with an increase in AC levels comparable to that observed by using flours from sources other than wheat,[~~57~~ 61] and some vegetable water extracts.[21]

Consistently with the literature,[~~58~~ 62] storage time significantly impacted AC, which decreased in all breads after 72 h of storage. However, *SFN100-T2* maintained a significantly higher value compared to *SFN0-T2* also in stored bread (~~ca.~~ approx. 76%), whereas no significant difference was observed between *SFN35-T2* and *SFN0-T2* (Table 2). Indeed, the smaller difference in AC levels between *SFN0-T2* and *SFN35-T2* compared to that between *SFN0-T2* and *SFN100-T2* could not be maintained after 72 h of bread storage.

The crumb and crust moisture of breads were significantly affected only by storage time (Table 2); consistently with the literature,[~~50~~ 54] both parameters significantly decreased in stored breads compared to fresh breads independently of the addition of SFN extract.

Overall, the use of an aqueous extract of SFN obtained with a controlled HC extraction process as a substitution ingredient for bread water (i.e., 35% and 100%) resulted in dough and bread samples with peculiar technological and antioxidant profiles.

The bread obtained with the SFN extract addition corresponding to the absolute threshold of taste perception (i.e., 35%), had the same sensory profile and specific volume of the control bread. Besides having a significant higher AC in the fresh bread, *SFN35-T2* also showed a higher alveograph dough extensibility – *L*, which is particularly relevant for ~~unrefined~~ WWF dough, as proven by the scientific studies trying to find strategies to enhance this parameter.[~~33~~,63,~~59~~,64] Furthermore, this sample had a different bread texture as regard to the hardness, springiness, and chewiness parameters, all of which had higher levels than those of the control bread.

The highest level of SFN extract supplementation determined a bread with a sensory profile clearly different than the control, hence this bread could be potentially included in the special bread category.[~~34,35~~ 36,37] *SFN100-T2* had the highest functional effect,[~~60~~ 65] since it exhibited the greatest AC in both fresh and stored breads. Furthermore, this sample showed improved rheological parameters, i.e. a significant higher alveograph dough extensibility – *L*, swelling index – *G*, and flour strength – *W*, and the highest bread specific volume, which are relevant results for ~~unrefined~~ WWF breads.[~~46~~ 50]

This study attempted to perform a first investigation of SFN extract produced with controlled HC as enrichment material for ~~unrefined~~ WWF bread. Both technological and antioxidant properties of bread enriched with SFN extract were evaluated. Several studies about bread enrichment with forest and agri-food by-products containing bioactive compounds mainly focused on the nutritional impact on bread; however, the technological issue should also be assessed to determine the suitability of the tested matrix for the development of attractive functional foods.[2,4,5,6] Results showed that SFN extract significantly increased the bread AC. Fresh breads enriched with SFN extract would be able to provide approximately 175 mg TEAC (35% SFN extract) and 229 mg TEAC (100% SFN extract) of antioxidants per bread portion (200 g), as compared to the 123 mg TEAC antioxidants provided by the control bread (0% SFN extract). Since bread is a staple food, consumed daily in several parts of the world, its contribution to the intake of valuable compounds in human diet is significant. Hence, even a small increase in the concentration of bread antioxidant compounds could significantly increase the total daily intake.[7]

Further research about the most sustainable and appropriate processing strategies and the most suitable kind of by-product to be supplemented should be explored to develop functional foods with nutritional, technological, and sensorial appeal. Indeed, the use of by-products in a dried form involves further processing treatments upstream. Hence, dried by-products, although having a higher stability during storage, can be considered less environmentally sustainable compared to the use of analogous materials in an unprocessed form such as aqueous solutions. Furthermore, controlled HC allowed to perform a green and affordable extraction process using water as the only solvent. High speed and efficiency are other important factors that characterise the HC process, especially considering that a low amount of raw material was used ~~(0.90% w/w dm~~ 9.0 g/kg for dry SFN). Considering that the total volume of SFN extract produced with a single HC process (i.e., 130 L) complies with the amounts required at industrial scales and based on the proven straightforward scalability of HC processes using fixed cavitation reactors[17] this kind of extraction process could be particularly interesting for the production of novel foods in the baking industry.

4. Conclusions

The SFN (*Abies Aalba* Mill. needles) aqueous extract produced by means of controlled HC extraction, which is a fast, simple and green extraction method with high efficiency and straightforward

496 scalability, appeared particularly interesting for the production of added-value and functional
497 breads. The SFN extract was able to positively affect not only the bread AC but also dough and bread
498 technological quality, emphasising the importance to select the most suitable by-product in the
499 most sustainable and appropriate physical form to produce attractive functional breads. The
500 possibility to use an enrichment material produced with a green extraction method and in the form
501 of an aqueous solution is particularly relevant since most of the literature studies used dried
502 materials obtained with processing treatments that may produce considerable environmental
503 impacts.

504 Despite the positive results obtained in this study on ~~unrefined~~ WWF bread enriched with SFN
505 extract, further investigation is necessary to gain further insight into the chemical composition of
506 the extract, the molecular effects accounting for the observed macroscopic results, the bio-
507 accessibility and bioavailability of antioxidant compounds and the sensory evaluation of enriched
508 breads.

References

1. O'Shea, N., Arendt, E. K., & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science and Emerging Technologies*, *16*, 1–10. https://doi.org/10.1016/j.ifset.2012.06.002
2. Dziki, D., Ròzylo, R., Gawlik-Dziki, U., & Świeca, M. (2014). Current trends in the enhancement of antioxidant activity of wheat bread by the addition of plant materials rich in phenolic compounds. *Trends in Food Science & Technology*, *40*, 48–61. https://doi.org/10.1016/j.tifs.2014.07.010
3. Faustino, M., Veiga, M., Sousa, P., Costa, E. M., Silva, S., & Pintado M. (2019). Agro-Food Byproducts as a New Source of Natural Food Additives. *Molecules*, 24, 1056. https://doi.org/10.3390/molecules24061056
4. Melini, V., Melini, F., Luziatelli, F., & Ruzzi, M. (2020). Functional Ingredients from Agri-Food Waste: Effect of Inclusion Thereof on Phenolic Compound Content and Bioaccessibility in Bakery Products. *Antioxidants*, *9*, 1216.
5. Betoret, E., & Rosell, C. M. (2019). Enrichment of bread with fruits and vegetables: Trends and strategies to increase functionality. *Cereal Chemistry*, *97*, 9–19. https://doi.org/10.1002/cche.10204
6. Xu, J., Wang, W., & Li, Y. (2019). Dough properties, bread quality, and associated interactions with added phenolic compounds: A review. *Journal of Functional Foods*, *52*, 629–639. https://doi.org/10.1016/j.jff.2018.11.052
7. Koch, W. (2019). Dietary Polyphenols — Important Non-Nutrients in the Prevention of Chronic Noncommunicable Diseases. A Systematic Review. *Nutrients*, *11*, 1039.

8. Gawlik-Dziki, U., Dziki, D., Baraniak, B., & Rufa, L. (2009). The effect of simulated digestion *in vitro* on bioactivity of wheat bread with Tartary buckwheat flavones addition. *LWT – Food Science and Technology*, *42*, 137–143.

9. Das, L., Raychaudhuri, U., & Chakraborty, R. (2012). Supplementation of common white bread by coriander leaf powder. *Food Science and Biotechnology*, *21*, 425–433.

10. Świeca, M., Śeczyk, Ł., Gawlik-Dziki, U., & Dziki, D. (2014). Bread enriched with quinoa leaves – The influence of protein- phenolics interactions on the nutritional and antioxidant quality. *Food Chemistry*, *162*, 54–62. https ://doi.org/10.1016/j.foodc hem.2014.04.044

11. Plazzotta, S., Sillani, S., & Manzocco, L. (2018). Exploitation of lettuce waste flour to increase bread functionality: effect on physical, nutritional, sensory properties and on consumer response. *International Journal of Food Science and Technology*, *53*, 2290–2297. https://doi.org/10.1111/ijfs.13820

12. Colantuono, A., Ferracane, R., & Vitaglione, P. (2018). Potential bioaccessibility and functionality of polyphenols and cynaropicrin from breads enriched with artichoke stem. *Food Chemistry*, *245*, 838–844.

13. Lafarga, T., Gallagher, E., Bademunt, A., Viñas, I., Bobo, G., Villaró, S., & Aguiló-Aguayo, I. (2019). characteristics of bread containing broccoli co - products. *Journal of Food Processing and Preservation*, *43*, 13861. https://doi.org/10.1111/jfpp.13861

14. Cedola, A., Palermo, C., Centonze, D., Del Nobile, M.A., & Conte, A. (2020a) Characterization and Bio-Accessibility Evaluation of Olive Leaf Extract-Enriched “Taralli”. *Foods*, *9*, 1268.

15. Bolek, S. (2020). Olive stone powder: A potential source of fiber and antioxidant and its effect on the rheological characteristics of biscuit dough and quality. *Innovative Food Science & Emerging Technologies*, *64*, 102423. https://doi.org/10.1016/j.ifset.2020.102423

16. Đurović, S., Vujanović, M., Radojković, M., Filipović, J., Filipović, V., Gašićd, U., Tešiće, Ž., Maškovićf, P., & Zekovićb, Z. (2020). The functional food production: Application of stinging nettle leaves and its extracts in the baking of a bread. *Food Chemistry*, *312*, 126091. https://doi.org/10.1016/j.foodchem.2019.126091

17. Meneguzzo, F., Albanese, L., & Zabini, F. (2021). Hydrodynamic Cavitation in Beer and Other Beverage Processing. In K. Knoerzer & K. Muthukumarappan (Eds.), *Innovative Food Processing Technologies* (1st ed., pp. 369–394). Elsevier. https://doi.org/10.1016/b978-0-08-100596-5.23022-9

18. Sanchez-Zapata, E., Fuentes-Zaragoza, E., Fernandez-Lopez, J., Sendra, E., Sayas, E., Navarro, C., Pérez-Álvarez, J.A. (2009). Preparation of dietary fiber powder from tiger nut (Cyperus esculentus) milk ("Horchata") by products and its physicochemical properties. *Journal of Agricultural and Food Chemistry*, *57*, 7719–7725.

19. Hobbs, D. A., Ashouri, A., George, T. W., Lovegrove, J. A., & Methven, L. (2014). The consumer acceptance of novel vegetable-enriched bread products as a potential vehicle to increase vegetable consumption. *Food Research International*, *58*, 15–22. https ://doi. org/10.1016/j.foodr es.2014.01.038

20. López-Nicolás, R., Frontela-Saseta, C., González-Abellán, R., Barado-Piqueras, A., Perez-Conesa, D., & Ros-Berruezo, G. (2014). Folate fortification of white and whole-grain bread by adding Swiss chard and spinach. Acceptability by consumers. *LWT - Food Science and Technology*, *59*, 263–269. https ://doi.org/10.1016/j.lwt.2014.05.007

21. Baiano, A., Viggiani, I., Terracone, C., Romaniello, R., & Del Nobile, M. A. (2015). Physical and Sensory Properties of Bread Enriched with Phenolic Aqueous Extracts from Vegetable Wastes. *Czech Journal of Food Sciences*, *33*, 247–253. https://doi.org/10.17221/528/2014-CJFS

22. Cedola, A., Cardinali, A., D'Antuono, I., Conte, A., & Del Nobile, M. A. (2020b). Cereal foods fortified with by-products from the olive oil industry. *Food Bioscience* , *33*, 100490. https://doi.org/10.1016/j.fbio.2019.100490

23. Sahin, H. T., & Yalcin, O. U. (2017). Chemical Composition and Utilization of Conifer Needles-A Review. *Journal of Applied Life Sciences International*, *14*, 1–11. https://doi.org/10.9734/JALSI/2017/37076

24. Jyske, T., Järvenpää, E., Kunnas, S., Sarjala, T., Raitanen, J.-E., Mäki, M., Pastell, H., Korpinen, R., Kaseva, J., & Tupasela, T. (2020). Sprouts and Needles of Norway Spruce (*Picea abies* (L.) Karst.) as Nordic Specialty—Consumer Acceptance, Stability of Nutrients, and Bioactivities during Storage. *Molecules*, *25*, 4187.

25. Valerón, N. R., Vasquez, D. P., & Munk, R. (2021). The Pinaceae species, flavor attributes for new culinary spices. *International Journal of Gastronomy and Food Science*, *23*, 100306. https://doi.org/10.1016/j.ijgfs.2021.100306

26. Chemat, F., Abert Vian, M., Fabiano-Tixier, A. S., Nutrizio, M., Režek Jambrak, A., Munekata, P. E. S., Lorenzo, J. M., Barba, F. J., Binello, A., & Cravotto, G. (2020). A review of sustainable and intensified techniques for extraction of food and natural products. *Green Chemistry*, *22*, 2325–2353. https://doi.org/10.1039/c9gc03878g

27. Albanese, L., Ciriminna, R., Meneguzzo, F., & Pagliaro, M. (2017). Beer-brewing powered by controlled hydrodynamic cavitation: Theory and real-scale experiments. *Journal of Cleaner Production*, *142*, 1457–1470. https://doi.org/10.1016/j.jclepro.2016.11.162

28. Ciriminna, R., Albanese, L., Di Stefano, V., Delisi, R., Avellone, G., Meneguzzo, F., & Pagliaro, M. (2018). Beer produced via hydrodynamic cavitation retains higher amounts of xanthohumol and other hops prenylflavonoids. *LWT - Food Science and Technology*, *91*, 160–167. https://doi.org/10.1016/j.lwt.2018.01.037

29. Meneguzzo, F., Brunetti, C., Fidalgo, A., Ciriminna, R., Delisi, R., Albanese, L., Zabini, F., Gori, A., dos Santos Nascimento, L. B., De Carlo, A., Ferrini F., Ilharco, L. M., Pagliaro, M. (2019). Real-Scale Integral Valorization of Waste Orange Peel via Hydrodynamic Cavitation. *Processes*, *7*, 581.

30. Albanese, L., Bonetti, A., D'Acqui, L. P., Meneguzzo, F., & Zabini, F. (2019). Affordable Production of Antioxidant Aqueous Solutions by Hydrodynamic Cavitation Processing of Silver Fir (*Abies alba* Mill.) Needles. *Foods*, *8*, 65. https://doi.org/10.3390/foods8020065

31. Nuzzo, D., Cristaldi, L., Sciortino, M., Albanese, L., Scurria, A., Zabini, F., Lino, C., Pagliaro, M., Meneguzzo, F., Di Carlo, M., Ciriminna, R. (2020). Exceptional Antioxidant, Non-Cytotoxic Activity of Integral Lemon Pectin from Hydrodynamic Cavitation. *Chemistry Select*, *5*, 5066–5071. https://doi.org/10.1002/slct.202000375

32. Pawar, S. K., Mahulkar, A. V., Pandit, A. B., Roy, K., & Moholkar, V. S. (2017). Sonochemical Effect Induced by Hydrodynamic Cavitation: Comparison of Venturi/Orifice Flow Geometries. *AIChE Journal*, 63, 4705–4716. https://doi.org/10.1002/aic

33. Carpenter, J., Badve, M., Rajoriya, S., George, S., Saharan, V. K., & Pandit, A. B. (2017). Hydrodynamic cavitation: An emerging technology for the intensification of various chemical and physical processes in a chemical process industry. *Reviews in Chemical Engineering*, 33, 433–468. https://doi.org/10.1515/revce-2016-0032

34. Cravotto, G., Mariatti, F., Gunjevic, V., Secondo, M., Villa, M., Parolin, J., & Cavaglià, G. (2018). Pilot scale cavitational reactors and other enabling technologies to design the industrial recovery of polyphenols from agro-food by-products, a technical and economical overview. *Foods*, 7, 1–14. https://doi.org/10.3390/foods7090130

35. Pagani, M. A., Marti, A., & Bottega, G. (2014). Wheat milling and flour quality evaluation. *Bakery products science and technology*, 17–53.

36. Rosell, C. M. (2012). The nutritional enhancement of wheat flour. In S. P. Cauvain (Ed.), *Bread making; improving quality* (2nd ed., pp. 687–710). Cambridge, UK: Woodhead Publishing Ltd.

37. Cauvain, S. (2015). *Technology of breadmaking*. New York: Springer Sciences.

38. Bordes, J., Branlard, G., Oury, F. X., Charmet, G., & Balfourier, F. (2008). Agronomic characteristics, grain quality and flour rheology of 372 bread wheats in a worldwide core collection. *Journal of Cereal Science*, *48*, 569–579. https://doi.org/10.1016/j.jcs.2008.05.005

39. Parenti, O., Guerrini, L., Cavallini, B., Baldi, F., & Zanoni, B. (2020a). Breadmaking with an old wholewheat flour: Optimization of ingredients to improve bread quality. *LWT - Food Science and Technology*, *121*, 108980. https://doi.org/10.1016/j.lwt.2019.108980

40. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT - Food Science and Technology*, *28*, 25–30 https://doi.org/10.1016/S0023-6438(95)80008-5

41. Zielinski, H., Michalska, A., Ceglijska, A., & Lamparski, G. (2008). Antioxidant properties and sensory quality of traditional rye bread as affected by the incorporation of four with different extraction rates in the formulation. *European Food Research and Technology*, *226*, 671–680. https://doi.org/10.1007/s00217-007-0576-1

42. Meilgaard, M. C., Carr, B. T., & Civille, G. V. (2006). *Sensory evaluation techniques*. CRC press.

43. Harris, E. K. (1960). Analysis of Experiments Measuring Threshold Taste. *International Biometric Society*, *16*, 245–260.

44. Gonázlez Viñas, M. A., & Salvador, M. D. (1998). Comparison of two simple methods for the measurement of detection thresholds for basic, umami and metallic tastes. *Journal of Sensory Studies*, *13*, 299–314. https://doi.org/10.1111/j.1745-459x.1998.tb00091.x

45. Law Decree 30.6.03, 196.

46. Sahi, S. S. (2014). Ascorbic acid and redox agents in bakery systems. *Bakery products science and technology*, 183-197

47. Zhang, L., Cheng, L., Jiang, L., Wang, Y., Yang, G., & He, G. (2010). Effects of tannic acid on gluten protein structure, dough properties and bread quality of Chinese wheat. *Journal of the Science of Food and Agriculture*, *90*, 2462–2468. https://doi.org/10.1002/jsfa.4107

48. Zhu, F., Sakulnak, R., & Wang, S. (2016). Effect of black tea on antioxidant, textural, and sensory properties of Chinese steamed bread. *Food Chemistry*, *194*, 1217–1223. https://doi.org/10.1016/j.foodchem.2015.08.110

49. Ning, J., Hou, G. G., Sun, J., Wan, X., & Dubat, A. (2017). Effect of green tea powder on the quality attributes and antioxidant activity of whole-wheat flour pan bread. *LWT - Food Science and Technology*, *79*, 342–348. https://doi.org/10.1016/j.lwt.2017.01.052

50. Parenti, O., Guerrini, L., & Zanoni, B. (2020b). Techniques and technologies for the breadmaking process with unrefined wheat flours. *Trends in Food Science & Technology*, *99*, 152–166. https://doi.org/10.1016/j.tifs.2020.02.034

51. Świeca, M., Gawlik-Dziki, U., Dziki, D., Baraniak, B., & Czyz, J. (2013). The influence of protein – flavonoid interactions on protein digestibility in vitro and the antioxidant quality of breads enriched with onion skin. *Food Chemistry*, *141*, 451–458. https://doi.org/10.1016/j.foodchem.2013.03.048

52. Zhu, F. (2015). Interactions between starch and phenolic compound. *Trends in Food Science & Technology*, *43*, 129–143. https://doi.org/10.1016/j.tifs.2015.02.003

53. Xu, J., Li, Y., Zhao, Y., Wang, D., & Wang, W. (2021). Influence of antioxidant dietary fiber on dough properties and bread qualities: A review. *Journal of Functional Foods*, *80*, 104434. https://doi.org/10.1016/j.jff.2021.104434

54. Fadda, C., Sanguinetti, A. M., Del Caro, A., Collar, C., & Piga, A. (2014). Bread Staling: Updating the View. *Comprehensive Reviews in Food Science and Food Safety*, *13*, 473–492. https://doi.org/10.1111/1541-4337.12064

55. Curti, E., Carini, E., Tribuzio, G., & Vittadini, E. (2015). Effect of bran on bread staling: Physico-chemical characterization and molecular mobility. *Journal of Cereal Science*, *65*, 25–30. https://doi.org/10.1016/j.jcs.2015.06.002

56. Filipčev, B., Lević, L., Bodroža-Solarov, M., Mišljenović, N., & Koprivica, G. (2010). Quality Characteristics and Antioxidant Properties of Breads Supplemented with Sugar Beet Molasses-Based Ingredients. *International Journal of Food Properties*, *5*, 1035–1053. https://doi.org/10.1080/10942910902950526

57. Prokopov, T., Chonova, V., Slavov, A., Dessev, T., Dimitrov, N., & Petkova, N. (2018). Effects on the quality and health-enhancing properties of industrial onion waste powder on bread. *Journal of Food Science and Technology*, *55*, 5091–5097. https://doi.org/10.1007/s13197-018-3448-8

58. Benítez, V., Esteban, R. M., Moniz, E., Casado, N., Aguilera, Y., & Esperanza, M. (2018). Breads fortified with wholegrain cereals and seeds as source of antioxidant dietary fibre and other bioactive compounds. *Journal of Cereal Science*, *82*, 113–120. https://doi.org/10.1016/j.jcs.2018.06.001

59. Vasileva, I., Denkova, R., Chochkov, R., Teneva, D., Denkova, Z., Dessevc, T., Deneve, P., & Slavov, A. (2018). Effect of lavender (Lavandula angustifolia) and melissa (Melissa Officinalis) waste on quality and shelf life of bread. *Food Chemistry*, *253*, 13–21. https://doi.org/10.1016/j.foodchem.2018.01.131

60. Niño-Medina, G., Muy-Rangel, D., De La Garza, A. L., Rubio-Carrasco, W., Pérez-Meza, B., Araujo-Chapa, A. P., Gutiérrez-Álvarez, K. A., & Urías-Orona, V. (2019). Dietary fiber from

chickpea (cicer arietinum) and soybean (glycine max) husk byproducts as baking additives: Functional and nutritional properties. *Molecules*, *24*, 991. https://doi.org/10.3390/molecules24050991

61. Dall'Asta, C., Cirlini, M., Morini, E., Rinaldi, M., Ganino, T., & Chiavaro, E. (2013). Effect of chestnut fl our supplementation on physico-chemical properties and volatiles in bread making. *LWT - Food Science and Technology*, *53*, 233–239. https://doi.org/10.1016/j.lwt.2013.02.025

62. Paciulli, M., Rinaldi, M., Cirlini, M., Scazzina, F., & Chiavaro, E. (2016). Chestnut flour addition in commercial gluten-free bread: A shelf-life study. *LWT - Food Science and Technology*, 70, 88–95. https://doi.org/10.1016/j.lwt.2016.02.034

63. Parenti, O., Carini, E., Marchini, M., Grazia, M., Guerrini, L., & Zanoni, B. (2021). Wholewheat bread: Effect of gradual water addition during kneading on dough and bread properties. *LWT - Food Science and Technology*, *142*, 111017. https://doi.org/10.1016/j.lwt.2021.111017

64. Guerrini, L., Parenti, O., Angeloni, G., & Zanoni, B. (2019). The bread making process of ancient wheat: A semi-structured interview to bakers. *Journal of Cereal Science*, *87*, 9–17. https://doi.org/10.1016/j.jcs.2019.02.006

65. Siró, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance-A review. *Appetite*, *51*, 456–467. https://doi.org/10.1016/j.appet.2008.05.060

Figure Legends

Figure 1. Evolution of temperature (black circles) and Cavitation Number (blue squares) during the HC-based extraction process.

Figure 2. The alveograph dough extensibility – *L* (mm), the dough swelling index – *G* (mm), and the flour strength – *W* (10^{-4} J) of *T2* dough samples supplemented with different levels of SFN extract: 0%, 35%, 100% (w/water w). Bars marked with different superscripts are significantly different at $p < 0.05$ significance level. Black bars represent the standard deviations.

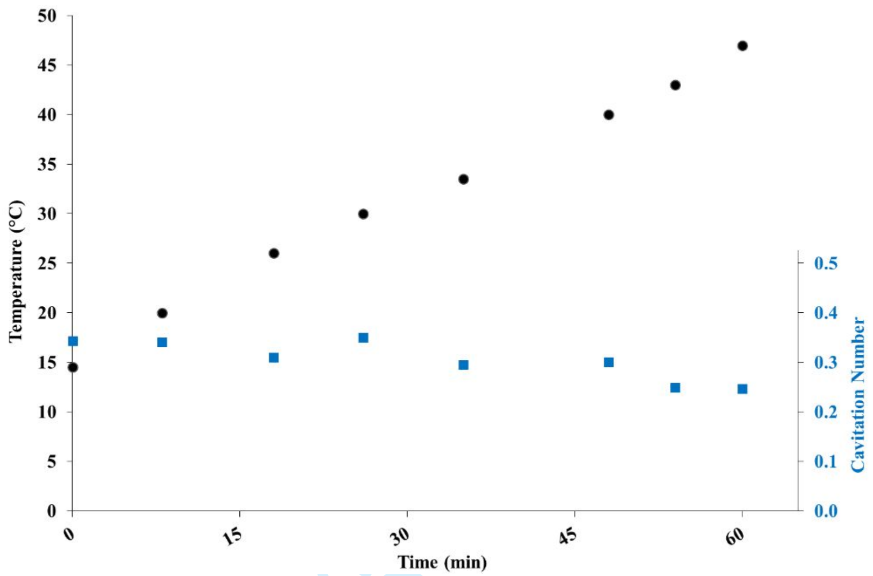
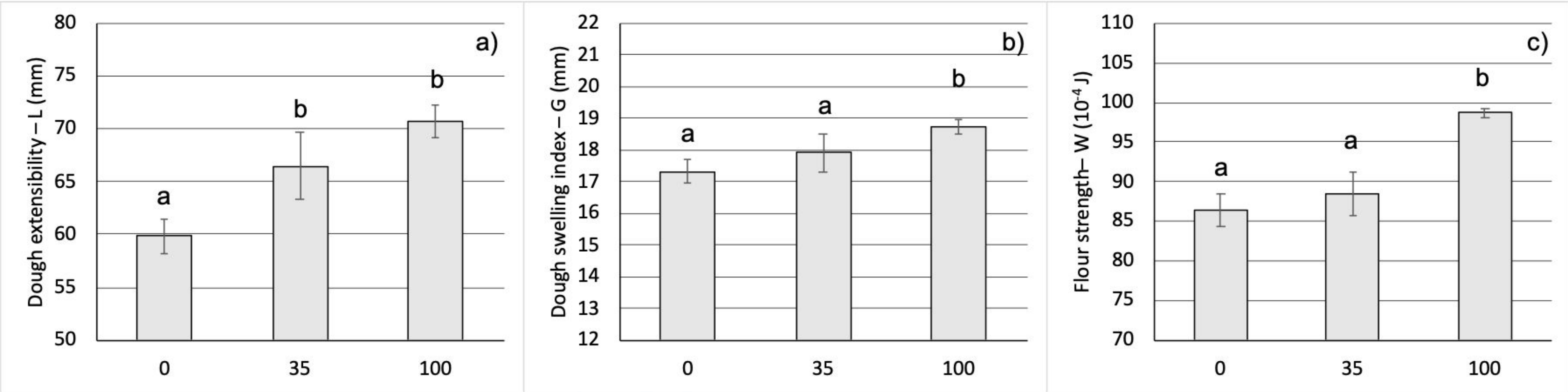

50
45
40
35
30
25
20
15
10
5
0
Temperature (°C)
0.5
0.4
0.3
0.2
0.1
0.0
Cavitation Number
0
15
30
45
60
Time (min)

Figure 1



Dough extensibility – L (mm)
80
75
70
65
60
55
50
a)
a
b
b
0
35
100
Dough swelling index – G (mm)
22
21
20
19
18
17
16
15
14
13
12
b)
a
a
b
0
35
100
Flour strength– W (10^{-4} J)
110
105
100
95
90
85
80
75
70
c)
a
a
b
0
35
100

Figure 2

Table 1 Alveographic parameters of T2 dough samples tested in the experimental trial 2 (*T2*)

Dough sample	***P*** **(mm H_2O)**	***L*** **(mm)**	***G*** **(mm)**	***W*** **(10^{-4} J)**	***P/L***	***Ie***
SFN0-T2	33.67 ± 2.08^a	59.83 ± 1.65^a	17.33 ± 0.35^a	86.33 ± 2.08^a	0.54 ± 0.07^a	62.53 ± 0.91^a
SFN35-T2	34.56 ± 0.35^a	66.46 ± 3.21^b	17.91 ± 0.59^a	88.50 ± 2.70^a	0.50 ± 0.03^a	61.98 ± 1.12^a
SFN-100T2	34.67 ± 1.00^a	70.67 ± 1.53^b	18.73 ± 0.21^b	98.67 ± 0.58^b	0.49 ± 0.02^a	63.17 ± 0.55^a
p SFN extract	ns	**	*	***	n.s.	n.s.

Data are expressed as mean ± standard deviation. *SFN0-T2* corresponded to dough sample with the addition of 0% silver fir needles (SFN) extract addition (w/water w), *SFN35-T2* to dough sample with 35% SFN extract addition (w/water w), and *SFN100-T2* to dough sample with 100% SFN extract addition (w/water w). *p* SFN extract refers to the main effect of the dough supplementation with SFN extract. *, **, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; n.s. indicates no significant differences at $p < 0.05$. Means in a column marked with different superscripts are significantly different ($p < 0.05$) as a function of SFN extract variable.

Table 2 Bread quality parameters of ~~*T2*~~ bread samples tested in the experimental trial 2 (*T2*)

Parameter	**Fresh bread samples**			**Stored bread samples (72 h)**			***p* SFN extract**	***p* Storage time**	***p* SFN extract*storage time**
	SFN0-T2	***SFN35-T2***	***SFN100-T2***	***SFN0-T2***	***SFN35-T2***	***SFN100-T2***			
Bread specific volume (L/kg)	3.267 ± 0.049^{a}	3.342 ± 0.067^{ab}	3.460 ± 0.021^{b}	n.d.	n.d.	n.d.	*	n.d.	n.d.
Crumb moisture ~~(%)~~ (g/kg)	~~46.97 ± 0.23^{x}~~ 469.7 ± 2.3^{x}	~~47.00 ± 0.32^{x}~~ 470.0 ± 3.2^{x}	~~46.97 ± 0.08^{x}~~ 469.7 ± 0.8^{x}	~~46.67 ± 0.17^{y}~~ 466.7 ± 1.7^{y}	~~46.59 ± 0.39^{y}~~ 465.9 ± 3.9^{y}	~~46.42 ± 0.39^{y}~~ 464.2 ± 3.9^{y}	ns	**	n.s.
Crust moisture ~~(%)~~ (g/kg)	~~24.53 ± 0.20^{x}~~ 245.3 ± 2.0^{x}	~~23.84 ± 0.72^{x}~~ 238.4 ± 7.2^{x}	~~23.89 ± 0.71^{x}~~ 238.9 ± 7.1^{x}	~~21.04 ± 0.36^{y}~~ 210.4 ± 3.6^{y}	~~20.52 ± 0.80^{y}~~ 205.2 ± 8.0^{y}	~~20.92 ± 0.36^{y}~~ 209.2 ± 3.6^{y}	ns	***	n.s.
Hardness (N)	3.78 ± 0.73^{ax}	5.18 ± 0.77^{bx}	3.13 ± 0.63^{ax}	4.28 ± 0.19^{cy}	6.11 ± 0.84^{dy}	4.20 ± 0.77^{cy}	***	*	n.s.
Cohesiveness	0.471 ± 0.021^{x}	0.562 ± 0.033^{x}	0.498 ± 0.052^{x}	0.305 ± 0.037^{y}	0.308 ± 0.043^{y}	0.303 ± 0.030^{y}	ns	***	n.s.
Springiness (mm)	0.949 ± 0.042^{ax}	1.152 ± 0.071^{bx}	0.914 ± 0.055^{ax}	0.814 ± 0.087^{cy}	0.736 ± 0.081^{cy}	0.762 ± 0.040^{cy}	*	***	**
Chewiness (mm*N)	1.282 ± 0.413^{ax}	1.802 ± 0.098^{bx}	1.133 ± 0.307^{ax}	0.761 ± 0.159^{cy}	0.879 ± 0.222^{dy}	0.766 ± 0.237^{cy}	**	**	n.s.
Antioxidant capacity (µmol TEAC/g dm)	0.613 ± 0.058^{ax}	0.873 ± 0.049^{bx}	1.144 ± 0.067^{cx}	0.580 ± 0.069^{dy}	0.715 ± 0.068^{edy}	0.730 ± 0.039^{ey}	*	*	n.s.

Data are expressed as mean ± standard deviation. *SFN0-T2* corresponded to bread sample with the addition of 0% silver fir needles (SFN) extract ~~addition~~ (w/water w), *SFN35-T2* to bread sample with 35% SFN extract addition (w/water w), and *SFN100-T2* to bread sample with 100% SFN extract addition (w/water w). *p* SFN extract refers to the main effect of the bread supplementation with SFN extract, *p* storage time to the main effect of the storage time, and *p* SFN*storage time to the interaction between the two independent variables. *, **, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; n.s. indicates no significant differences at $p < 0.05$ and n.d. indicates not determined parameters. Means in a raw marked with different superscripts are significantly different ($p < 0.05$). Specifically, “a”, “b”, “c”, “d”, and “e” refer to the main effect of SFN extract addition, “x” and “y” to the main effect of the storage time.