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Hemp (*Cannabis sativa* subsp. *sativa*) flour and protein preparation as natural nutrients and structure forming agents in starch based gluten-free bread

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ABSTRACT

The aim of the study was to use hemp flour and hemp protein concentrate as natural nutritional and structure-forming agents in gluten-free starch bread and to determine their impact on rheological properties of dough and quality and staling rate of bread crumb. The influence of hemp preparations on rheological characteristics of gluten-free dough was significant. Replacing a portion of starch with hemp flour resulted in a weakening of dough structure, which became more susceptible to deformation, while 20% share of hemp protein concentrate reinforced the structure of the tested dough. The presence of both investigated hemp preparations significantly improved nutritional value of bread. The changes involved increased levels of fiber from 15.2 up to 61.0 g/kg, and dietary fiber from 29.3 to 90.0 g/kg. Supplementation of bread with hemp protein favorably influenced the color of crumb by reducing its lightness from 62.3 to 40.8 and increased bread volume from 633 to 878 mL. Bread enriched with hemp preparations was characterized by improved sensory acceptance, especially in respect to color and flavor. Supplementation of starch bread with preparations based on hemp limited crumb hardening and amylopectin recrystallization during storage.

1. Introduction

Gluten-free market is one of the most dynamically developing food markets. This is due to increasing number of cases of food allergies and other diseases requiring gluten-free diet, better diagnostics and consumer confidence that gluten-free products are healthier. Many studies have been focused on quality improvements of gluten-free bread, but an important aspect is a development of highly nutritive recipes, because gluten-free products often have a poorer nutritional value than their gluten counterparts. Therefore, studies on gluten-free food require a holistic approach to the subject, which should take into account both quality and nutritional value (Matos & Rosell, 2015; Witczak, Ziobro, Juszczak, & Korus, 2016).

The paper aims to use hemp (*Cannabis sativa* L.) flour and protein preparation to enrich the recipe for gluten-free bread. Unlike *Cannabis sativa* subsp. *indica* leaf containing approx. 200 g/kg of psychoactive Δ9-tetrahydrocannabinol (THC), non-drug variety of *Cannabis sativa* subsp. *sativa*, containing approx. 3 g/kg THC, which is called hemp, could be legally cultivated in some countries (Girgih, Udenigwe, & Aluko, 2011). In most European countries the maximum THC content in legally grown cannabis is 2 g/kg on dry basis (Petrović, Debeljak, Kezić, & Džidara, 2015). Hempseeds contain substantial amounts of protein (200–250 g/kg), carbohydrates (200–300 g/kg), oil (250–350 g/kg) and insoluble fiber (100–150 g/kg) and significant level of minerals (56–80 g/kg) (Hofmanová, Švec, & Hrušková, 2014).

Hemp protein consists primarily of albumin and edestin (edistin), rich in essential amino acids (Callaway, 2004). Hempseed oil, which contains 80% polyunsaturated fatty acids (PUFAs), is a very rich source of linoleic and α-linolenic acids (Callaway, 2004; Teh & Birch, 2013). In Asian countries hempseeds had been used for centuries for culinary purposes or in folk medicine. Hempseeds are also used as food in Eastern Europe, mostly in Baltic states like Latvia (Callaway, 2004).

The aim of the study was to evaluate a possibility of using flour

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and protein preparation of hemp seed as a structure-enhancing ingredients in gluten-free starch bread. The effects of the partial replacement of starch (main raw material) in the basic formulations were investigated. The analyses were focused on rheological properties of the dough and contents of selected nutrients, quality of the resulting bread and the impact of the formula on the aging rate.

2. Materials and methods

2.1. Materials

The material consisted of corn starch (Bezgluten, Posądza, Poland), potato starch (Pepees S.A., Łomża, Poland), guar gum (Lotus Gums & Chemicals, Jodhpur, India), pectin (Pektowin, Jasło, Poland), freeze dried yeast Saf-instant (S.I. Lesaffre, Strasbourg, France), sucrose, salt, plant oil and water. Organic hemp flour (protein 299 g/kg, fat 81 g/kg, fiber 490 g/kg, carbohydrates 31 g/kg, according to the producer) and hemp protein concentrate (protein 489 g/kg, fat 116 g/kg, fiber 139 g/kg, carbohydrates 73 g/kg, according to the producer) were obtained from Radgeb company, Wrocław, Poland.

2.2. Methods

2.2.1. Formulation for gluten-free dough

The dough used for baking of gluten-free bread contained following ingredients: corn starch 480 g, potato starch 120 g, guar gum 10 g, pectin 10 g, freeze dried yeast 30 g, sucrose 12 g, salt 11 g, plant oil 18 g, water 570 g (Korus, Witczak, Ziobro, & Juszczak, 2015). Protein concentrate or hemp flour were used in exchange for 10 or 20% of the applied starches (60 or 120 g).

2.2.2. Rheological properties of dough

Rheological properties of the dough were determined at 25 °C using MARS II rheometer (Thermo-Haake, Karlsruhe, Germany) equipped with a system of parallel plates (diameter 35 mm, distance 1 mm).

Mechanical spectra were determined in a range of linear viscoelasticity at constant strain amplitude ($\gamma = 0.0005$) in a range of angular frequency 1–100 rad s^{-1}. Experimental data were fitted to power law model:

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

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

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

Creep and recovery tests were performed at constant strain in creep phase $\sigma_0 = 2$Pa during 150 s and 300 s of recovery phase. Experimental data were described using Burgers' model, which results in the following equations describing individual phases:

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

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

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

2.2.3. Bread baking

All dough ingredients were mixed for 8 min (Laboratory Spiral Mixer SP 12, Diosna, Osnabrück, Germany), next fermented for 15 min (35 °C, 80% relative humidity) and re-mixed. Then the portions of 250 g were weighed into greased pans. Final fermentation continued for 20 min. The loaves were baked in two independent batches, each containing 5 loaves, for 30 min at 230 °C in a convection oven MIWE Condo type CO 2 0608 (MIWE GmbH, Arnstein, Germany). After removing from pans bread was cooled down for 2 h at ambient temperature and used for further analyses or wrapped in polyethylene bags and stored in a cabinet at 22 ± 2 °C.

2.2.4. Bread analyses

Bread chemical composition determination included: protein (Nx6.25) content by Kjeldahl method (AOAC 950.36), crude fat content by Soxhlet method (AOAC 935.38), ash content (AOAC 923.03), total, soluble and insoluble dietary fiber content by enzymatic-gravimetric method AOAC 991.43 (AOAC, 2006).

Bread volume was measured using Volscan profiler 600 (Stable Micro Systems, Surrey, England). Image analysis was performed with the help of ImageJ software v. 1.44c (NIH, Bethesda, USA), evaluating porosity, cell density and percentage of pores>5 mm (Ziobro, Korus, Witczak, & Juszczak, 2012).

Analysis of crumb color in CIE L*a*b* system was performed by reflectance method using Color i5 spectrometer (X-Rite, Grand Rapids, USA; geometry d/8, illuminant D65, observer 10°).

Sensory analysis of bread was performed by the panel consisting of 14 experts with established sensory sensitivity and trained. The methods of sensory evaluation was based on the acceptance analysis of encoded bread samples on the seven-point scale, where 1 means "extremely dislike" and 7 – "extremely like". The analysis included the following quality attributes: overall appearance, structure and porosity, color, smell and taste.

Texture profile analysis (TPA) of bread crumb was performed, using texture analyzer TA-XT2plus (Stable Micro Systems, Surrey, England). Sample of bread crumb with a height 2 cm was pressed at the compression rate 5 mm/s to reach 50% deformation in two cycles with 5 s waiting time between the cycles. The resulting hardness, cohesiveness and chewiness of the crumb were used as indicators of textural changes during storage. The analysis were performed after 2, 24 and 48 h after baking.

Thermal properties of gluten-free bread crumb were characterized by means of differential scanning calorimeter DSC 204F1 Phoenix (Netzsch-Gerätebau, Selb, Germany). Crumb samples after 2, 24 and 48 h after baking were closed hermetically in aluminum pans and heated from 25 to 100 °C at a rate of 10 °C/min. Temperatures and enthalpy of thermal transitions were determined with the use of Proteus Analysis software (Netzsch-Gerätebau, Selb, Germany). Enthalpy values were expressed as J/g of dry basis of crumb.

2.2.5. Statistical analysis

All analyses were performed in 3 replicates, unless stated otherwise. In order to establish the statistical differences between means, the data were treated by one-factor analysis of variance, and the least significant difference (LSD) at significance level 0.05 was calculated using Fisher post hoc test. In order to determine both the influence of added preparation and its addition level, as well as time, two or three way ANOVA was applied. The dependencies between analyzed parameters were identified using the values of Pearson's correlation coefficients. The calculations were performed using statistical software package Statistica 8.0 (StatSoft Inc., Tulsa, USA).