Fizikalno-kemijska svojstva kruha sa dodatkom crnog češnjaka

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GRADUATE THESIS

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PHYSICOCHEMICAL PROPERTIES OF BLACK GARLIC BREAD

Experimental part of Graduate thesis was done at the Department of Animal Products Technology and Quality Management, Wrocław University of Environmental and Life Sciences under the supervision of Małgorzata Korzeniowska, Full Professor.

Theoretical part of Graduate thesis was done in the Section for Food Preparation Processes at the Department of Food Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb under the supervision of Suzana Rimac Brnčić, Full Professor, University of Zagreb.

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Fizikalno-kemijska svojstva kruha sa dodatkom crnog češnjaka

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Sažetak: Kruh se sve više obogaćuje različitim dodacima, uključujući češnjak koji ima oštar miris te ga je moguće zamijeniti crnim češnjakom. U ovom radu je ispitivana praktična primjena praha crnog češnjaka u proizvodnji kruha od pšeničnog brašna. Cilj je bio odrediti utjecaj dodatka praha crnog češnjaka (3; 6 i 12%) na fizikalna, kemijska i senzorska svojstva kruha. Ispitivana svojstva su: specifični volumen, boja, poroznost, tekstura (tvrdoća, kohezivnost, gumenost, rastezljivost, žvakavost), udjel ukupnih polifenola određen Folin-Ciocalteau metodom, antioksidacijski kapacitet određen FRAP i ABTS metodama. Navedena svojstva su određena za svježi kruh i kruh nakon 24 sata. Prema rezultatima, kruh s dodatkom praha crnog češnjaka (3%) imao je najveći specifični volumen te je postigao najbolje rezultate za gotovo sve parametre teksture. Slični rezultati su postignuti i za senzorske parametre. Skladištenje nije imalo utjecaja na boju kruha, dok je na tvrdoću imalo veliki utjecaj.

Ključne riječi: crni češnjak, kruh, TPA, polifenoli, antioksidacijska aktivnost

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Abstract: Bread is increasingly enriched with various additives, including the garlic that has strong pungent odour so it can be replaced with black garlic. In this thesis, the practical application of black garlic powder in wheat flour bread production was evaluated. The aim was to determine the influence of black garlic powder addition (3; 6 and 12%) on physical, chemical and sensory characteristics of bread. Evaluated characteristics are: bread specific volume, colour, crumb porosity, texture (hardness, cohesiveness, gumminess, springiness, chewiness), total phenolic content determined using Folin-Ciocalteau method, antioxidant capacity using FRAP and ABTS methods. These characteristics were evaluated for the fresh bread and bread after 24 hours storage. According to the obtained results, bread prepared with 3% black garlic powder addition had the highest specific volume and achieved the best scores for almost all texture parameters. Similar results were achieved for sensory parameters. Storage did not have influence on bread colour, while on hardness it had big influence.

Keywords: black garlic, bread, TPA, polyphenols, antioxidant activity

Thesis contains: 41 pages, 7 figures, 7 tables, 65 references

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1. INTRODUCTION

During the past decade, changes in the understanding of the role of foods in human health were observed. Scientific studies have shifted from the primary role of food as the source of energy and forming substances to the action of biologically active food components. There has been growing interest in active role of food in promoting well-being and preventing diseases, such as cancer, cardiovascular diseases and osteoporosis (Grajek et al, 2005). According to that, food industry invests in technology development in order to produce functional foods. One of the ways to produce functional foods is to fortify traditional foods with bioactive substances, naturally occurring in food (Spence, 2006). Bread, as one of the oldest functional foods, has been investigated in many studies. Recent studies show that many beneficial ingredients such as phenolic antioxidants, dietary fibres and n-3 fatty acids can be used in bread production. This results in functional and healthy products, low in calories and cholesterol (Rahaie et al., 2014). Garlic (*Allium sativum L.*) belongs to the Alliaceae family and is a frequent ingredient in gastronomy. It shows a variety of bioactive effects, such as antioxidant, antihypertensive, antimicrobial, hepatoprotective, anticancer, and insecticidal properties. Those properties are linked to antioxidant polyphenolic and bioactive sulphur compounds, but when the garlic is crushed or damaged those bioactive sulphur compounds produce a strong pungent odour (Choi et al., 2014). Black garlic does not release a strong pungent odour due to reduced content of allicin. Allicin was converted into antioxidant compounds during the aging process (Kimura et al., 2017). Black garlic also shows more than 10-fold increase in antioxidant activity and 7-fold increase in polyphenol content, compared to the normal garlic (Kim et al., 2012a).

In this thesis, the practical application of black garlic powder in wheat flour bread will be evaluated. More precisely, 3%, 6% and 12% black garlic powder (w/w) will be added to the wheat flour and the physical and chemical characteristics of fresh bread and bread after 24 hours storage will be evaluated. Evaluated physical characteristics include bread specific volume, colour, crumb porosity, texture (hardness, cohesiveness, gumminess, springiness and chewiness). Evaluated chemical characteristics are total polyphenol content and antioxidant activity determined using FRAP and ABTS assays. Sensory analysis was conducted to evaluate external appearance, smell, texture, taste and overall liking of fresh bread and stored bread.

2. THEORETICAL PART

2.1. BREAD

Bread is one of the staple foods consumed by humanity and it is considered to be one of the oldest processed foods. It is not know the exact time bread was the first time made but it is likely that the place of discovery was in the Middle East (Cauvain, 2015). It belongs to the traditional diet, especially of the poor and it is the main food in European countries, as well as in America, North Africa and Middle East, whereas rice is the main food in East Asia (Kourkouta et al, 2017). Bread (Figure 1), as one of the most frequently eaten foods, is important source of carbohydrates, proteins and vitamins B and E. In the developed countries is noticed increasing consumption of breads prepared with whole grain and multigrain flours, this may be due to increased interest in healthy, balanced diet with reduced consumption of simple carbohydrates, fat and cholesterol but increased consumption of complex carbohydrates, dietary fibre and plant proteins (Peña, 2002).

Figure 1. White bread loaf (Anonymous, 2019)

2.1.1 Bread types

There are three types of bread: flat, leavened and steamed. All three of them are prepared from a refined flour-water dough but each type of bread has different processing conditions, end-product properties and grain quality needs.

Leavened breads are the most popular type of bread and are made with aerated, yeasted viscoelastic doughs. Those doughs are expanded by the gas produced by the yeast fermentation process in order to gain volume and decrease its density. Volume and density, as well as the size of the bread is determined by a combination of bread formula, length of fermentation stage, work used in bread-making dough and time bread dough spent rising before baking. Preferred type of grain for the manufacturing leavened breads are hard to medium-hard grains because the levels of damaged starch produced by these wheat classes are appropriate to achieve high dough water absorption. Hard to medium-hard wheats are also suitable for the mechanized production of leavened breads such as pan-type bread, hot-dog buns and hamburger buns because they yield strong flour doughs. Wheats that yield medium-strong doughs are suitable for the production of French-type and flat-type (Mexican flour *tortilla*, Indian *chapati*, etc.) bread, using semi-mechanized or manual processes. On the other hand, soft wheats which produce weak doughs are used for production of Asian steamed breads (Peña, 2002).

2.1.2. Basic ingredients and baking technology

The most important ingredients in bread production are flour and water and amount of flour added is always 100% while the rest of the ingredients are shown as the percent of the amount of flour by weight. Water should be added in about 50% since it results in finely textured, light bread. Other than flour and water, ingredients such as yeast, salt, sugar and shortening agents are also added in amounts of 2%, 2%, 4% and 3%, respectively.

Baker's yeast (trade name for the organism *Saccharomyces cerevisiae*) is used for fermentation of wheat flour sugars into moisture and CO_2 and as water vapour and CO_2 expand due to high temperature they prevent high rate of temperature rise of bread crumb and excessive moisture evaporation. Salt is added to strengthen the gluten and for converting yeast's activity of controlling expansion of the dough. Sugar is added for initiation of fermentation, whereas shortening is added for increasing the machinability and slicability. Due to growing consumer demand for high quality bread, mechanization and large scale production functional food additives (e.g., emulsifiers, anti-staling agents) are being added in the bread production. In a

large scale, industrial production is important addition of emulsifiers as their addition results in greater dough strength, improved hydration, crumb structure, slicing characteristics, gas holding capacity and extended shelf life (Mondal & Datta, 2008).

As a result of growing demand for high quality bread industrial production took over from the high variety bakeries and different baking technologies were developed to respond to new consumers' demands (Decock & Cappelle, 2005). In addition to that, new materials and methods are being introduced as well for production of better quality products, development of nutritionally superior and economically viable products.

Bakery products could be produced by three different methods. First one is straight dough method which means that mixing of all the ingredients is performed in one step. Second method is sponge and dough method where the mixing of the ingredients is performed in two steps. In the first step is prepared leavening agent by mixing yeast and certain amount of water and the mixture is left to develop for a few hours, after that it is added to the rest of the ingredients. Chorleywood method is the third method and in that method all the ingredients are mixed in an ultrahigh mixer for a few minutes. Fresh bread has short shelf life and it is suspected to number of chemical and physical changes, known as staling (Giannou et al., 2003).

2.1.4. Bread as functional food

As mentioned before, consumers are more and more interested in eating healthily and how foods affect well-being and prevent different types of diseases such as cardiovascular diseases. As a result, term "functional food" was coined in Japan in 1980s and it describes functional food as the food that together with the basic nutritional impact has beneficial effects on one or more functions of the human organism and decreases risk of evolution of different types of diseases. There are three types of functional foods (Grajek et al., 2005):

1) Conventional foods containing bioactive substances that are naturally present in a food (e.g., dietary fibre)
2) Foods fortified with bioactive substances (e.g., probiotics, antioxidants)
3) Synthesized food ingredients added to traditional foods (e.g., prebiotics)

Typical fortified food are cereal flours, such as wheat and maize flour, and the bread made from refined white flour is preferred by consumers to the one made from whole grain. But for the healthy lifestyle is recommended to consume more whole grain bread than white wheat bread (Youseff et al., 2014, Sullivan et al, 2011). Fortifying dough formulations with new ingredients can enhance bread quality and acceptability, with results in improvement of consumers' nutritional status and resistance to degenerative diseases associated with today's lifestyle (Poinot et al., 2009, Ruiz-Ruiz et al., 2015).

2.2. BLACK GARLIC

Garlic (*Allium sativum* L.) is one of food ingredients widely used in gastronomy, as well as medicinal plant for over 4000 years (Block, 1985). Biological and medical functions of garlic are mainly due to its organo-sulphur compounds (Augusti & Mathew, 1974). Primary sulphur-containing constituents are the S-alk(en)yl-L-cystein sulphoxides, such as alliin and γ-glutamylcysteins are important storage peptides and biosynthetic intermediates for ACSOs from which other compounds, such as allicin, diallyl sulphide (DAS), diallyl disulphide (DADS) and others, are synthesized (Lancaster & Shaw, 1989). Due to these compounds garlic has its characteristic odour and flavour, as well as most of its biological properties (Lanzotti, 2006).

Figure 2. Garlic during fermentation process (Kimura et al., 2017)

Black garlic is a product that is traditionally produced in Southeast Asia and recently introduced to other countries (Toledano-Medina et al, 2015). It is prepared by aging fresh garlic at the temperature in the range from 65°C to 90°C and a relative humidity of 60-80%. While processing it loses the strong odour of fresh garlic, this is because allicin is converted into water-soluble antioxidant compounds (Kim et al, 2012b). During processing on high temperatures fresh garlic undergoes intensive Maillard browning, in the beginning caramel and brown tones appear and after several days the garlic changes colour to black. Colour change during the fermentation process can be seen in Figure 2. On the end of aging process black garlic has a rubbery texture and bittersweet taste (Toledano-Medina et al, 2016). Several studies have reported beneficial health effects of black garlic, such as antioxidative, antiallergic, anti-inflammative, anticarcenogenic and antidiabetic effects (Jeong et al., 2016; Park et al., 2014; Ha et al., 2015; Kim et al., 2014; Yoo et al., 2014).

2.2.1. Changes during black garlic processing

Fresh garlic contains a high amount of γ-glutamylcysteines that can be hydrolysed and oxidized to form alliin, which naturally occurs during the storage of fresh garlic at a cool temperatures. After processing, alliinase lyses alliin to form alkyl alkane-thiosulfinates such as allicin (Amagase et al., 2001, Corto-Martinez et al., 2007). Those compounds are immediately degraded to the compounds such as diallyl sulfide, diallyl disulfide, diallyl trisulfide, dithiins and other (Corto-Martinez et al., 2007, Amagase, 2006).

During the aging process allicin is converted into water soluble antioxidant compounds such as S-allylcysteine, tetrahydro-β-carbolines, biologically active alkaloids and flavonoid-like compounds. S-allylcysteine is formed by the catabolism of γ-glutamylcysteine, whilst tetrahydro-β-carboline derivates are formed by condensation between tryptophan and aldehyde, which is similar to the production of pyruvic acid by the allin-allicin pathway or the Maillard reaction process (Corzo-Martínez et al, 2007; Ichikawa et al, 2002). Some chemical compounds formed during the thermal processing are key intermediate compounds of Maillard reactions. One of those compounds is 5-hydroxymethylfurfural (HMF), which is also one of the main antioxidant compounds in black garlic. Production of HMF is connected to the rate of black colour formation and changes in HMF content can be used as a monitoring index for predicting the rate of black colour formation in the samples. HMF production increased during the thermal processing and it is faster with the higher temperature.

Typical black colour of the black garlic is also formed by non-enzymatic browning reactions during the aging processes which greatly depends on the temperature, and those reactions can also lead to the formation of some antioxidant compounds. When the black garlic is produced at the temperature of 60°C its colour is not completely black, while processing at 90°C results in non-ideal tastes such as bitter and sour. The best quality black garlic has when it is produced at the temperature between 70°C and 80°C. At the temperature of 60°C aging speed of black garlic is slow and at 80°C to 90°C aging occurs smoothly, but the adequate condition is hard to find because of fluctuating phenol and reducing sugar content (Zhang et al., 2015).

Total phenols content increases in the black garlic compared with that in the fresh garlic and the increase in total phenols content improves antioxidant capacity of black garlic. The total phenols content increases during thermal processing and the increased rate is faster at higher temperatures. At the temperature of 60°C total phenols content was increasing during the processing which means that accumulation rate of total phenols exceeded its consumption rate. On the other hand, in the early stage of the processing at the temperature of 70°C, 80°C and 90°C total phenols content increases, but at the later stage it decreases. This indicates that the accumulation rate of total phenols was less than its consumption rate. The maximum of total phenols content is reached when the aging process is conducted at 80°C, while under 70°C total phenols content is also good (Zhang et al, 2015).

2.3. ANTIOXIDANTS

Antioxidants are compounds or substances, when present in low concentration compared to those of an oxidants, delay or inhibit oxidation of oxidasable substrates (Halliwell & Gutterige, 1989). Oxidants are normal product of aerobic metabolism but elevated rates of oxidants can be produced under pathophysiological conditions and an imbalance when more oxidants than antioxidants are present in the body is called ‘oxidative stress’ (Sies, 1997).

2.3.1. Types of biological antioxidants

2.3.1.1. Enzymes

Several enzymes have primary functions to decrease the amount of oxidants or potential oxidants. Some of the enzymes are superoxide dismutase, catalase, selenium glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase.

2.3.1.1.1. Superoxide dismutase

Superoxide dismutase catalyses the following reaction:

$$2\ O_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2 \qquad [1]$$

2.3.1.1.2. Catalase

Hydrogen peroxide is one of the products of the action of superoxide dismutase. It can be detoxified by several enzymes, including catalase which catalyses reaction:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2 \qquad [2]$$

In the presence of transition metals (e.g. copper or iron) and the absence of superoxide dismutase and catalase the extremely reactive hydroxyl radical OH• is formed in the following reaction:

$$O_2^{-}\bullet + Fe^{3+} \rightarrow O_2 + Fe^{2+} \qquad [3]$$

$$H_2O_2 + Fe^{2+} \rightarrow OH\bullet + OH^- + Fe^{3+} \qquad [4]$$

2.3.1.1.3. Selenium glutathione peroxidase (Se-GSH-Px)

Selenium glutathione peroxidase, together with phospholipase A_2 (PlaseA_2), convert potentially harmful phospholipid hydroperoxides (PLOOH) to free fatty acid hydroperoxides (LOOH) and lysophospholipids. Next step is converting those compounds to harmless fatty acid alcohols (LOH).

$$PLOOH \xrightarrow{\text{Plase}A_2} Lysophospholipid + LOOH \qquad [5]$$

$$LOOH + 2\ GSH \xrightarrow{Se-GSH-Px} LOH + GSSG + H_2O \qquad [6]$$

2.3.1.1.4. Phospholipid hydroperoxide glutathione peroxidase

Phospholipid hydroperoxide glutathione peroxidase is an enzyme that acts directly on phospholipid hydroperoxides and the reaction catalysed by PLOOH-GSH-Px is shown:

$$PLOOH + 2\,GSH \xrightarrow{PLOOH-GSH-Px} Phospholipid - LOH + GSSG + H_2O \qquad [7]$$

PLOOH-GSH-Px acting this way can remove the membrane-perturbing properties of phospholipid-LOOH without activating or mobilizing PLaseA_2 which can liberate excessive amounts of substrates for prostanoid synthesis. In addition, PLOOH-GSH-Px can reduce membrane-associated cholesterol hydroperoxides and by doing that it can diminish the amount of potentially harmful lipid hydroperoxides (Krinsky, 1992).

2.3.1.2. Non-enzymatic processes

A very large number of substances act like antioxidants and their antioxidative activity is determined by their ability to prevent lipid peroxidation or metal-catalysed radical reactions. Those compounds can be lipid-soluble or water-soluble. Some of the lipid-soluble antioxidants are tocopherols, carotenoids, quinones and bilirubin, while major water-soluble antioxidants are ascorbic acid, uric acid, metal-binding proteins and binding proteins for heme and heme-containing proteins (Krinsky, 1992).

2.3.1.2.1. Vitamin E

Mechanism of action of tocopherol is inactivation of two equivalents of chain-carrying peroxy radicals per molecule of inhibitor. Vitamin E can be regenerated through reaction of a reducing agent (e.g. vitamin C) at the lower radical concentrations. There are four major tocopherols and the most biologically active one is α-tocopherol (Larson, 1988).

2.3.1.2.2. Flavonoids

Flavonoids are naturally occurring compounds and they are the most common in plant leaves, flowering tissues and pollens, but are also abundant in stems and barks. Light affects synthesis of large number of flavonoids and other phenolic compounds and it is observed that plants grown in sun have higher levels of flavonoids of those grown in shade. It has also been observed that flavonoids, with absorption in the 300-400 nm UV region, act like internal light

filters for protection from UV damage. Some of the UV-absorbing compounds are present in high concentration in plant vacuoles of epidermal cells and they can be extracted with polar solvents, such as aqueous methanol. Compounds that act like light filters have strong antioxidant effects to provide protection against oxidative damage generated thermally or by light (Larson, 1988).

2.3.1.2.3. Alkaloids

Basic nitrogen compounds of higher plants include representatives which act like strong inhibitors of some oxidative processes in vivo and in vitro. Some alkaloids with different structural types are potent inhibitors of $^1O_2$ and very effective alkaloids in this group are indole alkaloids such as strychnine and brucine. Those alkaloids are physical quenchers and are not destroyed chemically which means they could inactivate many molecules of singlet oxygen per molecule of alkaloid. Tertiary amines (e.g. trimethylamine) are effective quenchers of peroxy radicals and the radicals derived from the amines have large termination rate, which means they are efficient in disruption of radical chain reactions (Larson, 1988).

2.3.1.2.4. β-carotene

B-carotene acts like powerful quencher of singlet oxygen and very low concentrations of β-carotene are needed for effective protection of membrane lipids from reactions of singlet oxygen which lead to peroxidation. Free radicals are also reactive to β-carotene (Larson, 1988).

2.3.1.2.5. Other compounds

Compounds such as phenolic acids (e.g. caffeic acid and chlorgenic acid), chlorophyll derivates (e.g. chlorophyll and pheophytin), amino acids and amines, vitamin C and others are considered good antioxidants. Chlorophyll and pheophytin are inhibitors of auto oxidation under dark conditions, while the amino acids with antioxidant activity are arginine, histidine, cysteine, tryptophan, lysine, methionine and threonine. Vitamin C is present in high concentrations in many cellular environments, such as chloroplasts, and it exerts antioxidative activity in way of the inhibition of the peroxy radical-initiated oxidation of methyl inoleate, as well as acting as a chain-breaking scavenger for peroxy radicals and synergist with vitamin E (Larson, 1988).

2.3.2. Mechanisms of action

Almost all biological lipids contain some polyunsaturated fatty acids (e.g. cholesteryl esters, phospholipids, triacylglycerols) which can be subjected to lipid peroxidation, initiated by free radicals. Therefore, lipid peroxidation is common a process, but because of the presence of antioxidants negative effects on body not shown. Antioxidant effects on lipid peroxidative reactions include several steps: initiation, propagation, singlet oxygen initiation, reinitiation and product removal.

There is a large number of radical species (e.g. hydroxyl radical (OH•), peroxyl radical (LOO•), alkoxyl radical (LO•)) that can initiate lipid peroxidation by abstracting one of the doubly allylic hydrogen atoms on the carbon atom between two double bonds. As a result of the radical attack on polyunsaturated fatty acid, a delocalized pentadienyl radical (L•) is formed. Pentadienyl radical is extremely reactive and when reacting with oxygen it forms the peroxyl radical LOO•. Peroxyl radical can then extract hydrogen atom from another unsaturated fatty acid and produce another free radical (L•) and a peroxide (LOOH). Singlet oxygen can initiate lipid peroxidation as well, even though it is usually limited to photosensitized reactions and it is formed either in eosinophils or from the reaction of ozone with biological material. As mentioned before, carotenoids are strong quenchers of singlet oxygen and can also react directly with radicals involved in lipid peroxidation. Peroxides already present in the body can be subjected to the lipid peroxidation by metal-catalysed breakdown. Metals that are part of this reaction are both oxidized and reduced transition metals, such as copper and iron. Those metals catalyse decomposition of peroxides to alkoxyl, alkyl or hydroxyl form which can then initiate the peroxidative process, described before. Reinitiation can be inhibited by decreasing the free concentration of iron or copper in the body or by removal of the hydroperoxide (LOOH). Hydroperoxide is removed by the action of several different enzymes, such as glutathione peroxidase, selenoperoxidase and phospoholipid hydroperoxide gluthatione peroxidase that catalyse the reduction of the hydroperoxide to the corresponding alcohol (LOH). Termination is the final step in which antioxidants react with the chain propagating radical species (peroxyl or alkoxyl radicals), and it results in the formation of species that are not capable of hydrogen abstraction (Krinsky, 1992).

3. EXPERIMENTAL PART

3.1. MATERIALS

In this work, dried and grounded black garlic powder has been used. The whole black garlic cloves (*Allium sativum* L.) were delivered by the company Farma Paszków (Warsaw, Poland) and dried using convection method. Dried black garlic cloves were grounded using a grinder to obtain the powder form.

3.1.1. Laboratory instruments equipment

- Digital scale (Radwag PS 2100.R1, North Miami Beach, USA)
- Analytical scale (Radwag AS 160/C/2, North Miami Beach, USA)
- TPA testing machine (ZWICK / ROELL Z010, Ulm, Germany)
- Ultrasonic cleaner (Sonic 6D, Polsonic, Warsaw, Poland)
- Universal laboratory mill WZ-1 (ZBPP, Bydgoszcz, Poland)
- UV-vis spectrophotometer (Shimadzu UV-2401 PC, Kyoto, Japan)
- Minolta colorimeter (CR-400/410, Konica Minolta, Japan)
- Volume meter (SA-WY device, ZBPP, Bydgoszcz, Poland)
- Laboratory oven (Brabender, Duisburg, Germany)
- Sigma mixer S 300 (Brabender farinograph, Duisburg, Germany)

3.1.2. Laboratory tools

- Laboratory glasses
- Whatman no.1 filter paper
- Cuvettes for spectrophotometer
- Test tubes
- Test tube racks
- Erlenmyer flask
- Volumetric flask (10 mL volume)
- Baking tins
- Pots
- Spoon
- Glass rods

- Spatula
- Micropipettes

3.1.3. Substrates and chemicals

- Water
- Distilled water
- Redistilled water
- Salt
- Wheat flour type T-650 (GoodMills Polska Sp. z o.o., Stradunia, Poland)
- Pressed Yeast (Lesaffre, Poland)
- Methanol, 80% (Archem, Wrocław, Poland)
- 1% HCl (Archem, Wrocław, Poland)
- Folin-Ciocalteu reagent (Archem, Wrocław, Poland)
- Sodium carbonate solution (Archem, Wrocław, Poland)
- Gallic acid (Archem, Wrocław, Poland)
- Acetate buffer (pH 3.6) (Archem, Wrocław, Poland)
- 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) (Fluka, Seelze, Germany)
- Iron(III)chloride hexahydrate pure p.a. (Chempur, Karlsruhe, Germany)
- 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Archem, Wrocław, Poland)
- 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Archem, Wrocław, Poland)
- Ethanol, 96% (Archem, Wrocław, Poland)
- 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Archem, Wrocław, Poland)
- Potassium persulfate (Archem, Wrocław, Poland)

3.2. METHODS

3.2.1. Baking bread

The doughs were prepared using the single-phase method in which black garlic powder was added directly to the mixture. For dough preparation was used 250 g of wheat flour type 650 to which was added 1.5% of salt and 3% of baker's yeast. Black garlic powder was added in the amounts of 3%, 6% and 12% and the control sample was wheat flour without added black garlic powder. Water was added in the amounts allowing to obtain 300 FU consistency. Wheat flour with different concentrations of black garlic powder was added into Sigma mixer S 300 (Brabender farinograph, Duisburg, Germany), yeast and salt were dissolved in the water and then added to the mixture. Blending time was 3 minutes. After that the dough was placed in the baking tin and fermented at 30°C and relative humidity about 85%. The dough was double kneaded by hand after 60 minutes and then after 30 minutes of fermentation. After the second kneading, the dough was left in the fermentation chamber. The dough was baked using laboratory oven (Brabender, Duisburg, Germany) for 30 minutes at 230°C.

3.2.2. Bread characteristics

3.2.2.1. Bread specific volume

Bread volume was measured after the bread has cooled down and after one day of storage, using the standard millet displacement method (AACC, 2000). Seed displacement method determines the volume of oddly shaped baked products from the volume of seeds they displace (Whitaker & Barringer, 2004). Measurements were conducted by placing bread loaves in four different directions in the SA-WY device (ZBPP, Bydgoszcz, Poland). Specific volume was obtained by dividing bread volume with bread weight and the results are shown as cm^3 per 100 grams.

3.2.2.2. Bread colour

Bread crumb colour was determined after cooling down and after one day of storage. Colour was measured using Minolta Colorimeter (CR-400/410, Konica Milnota, Japan) on three different points of the slice (crumb), with L ($L = 0$ [black] and $L = 100$ [white]), a* ($-a$ = greenness and $+a$ = redness), b* ($-b$ = blueness and $+b$ = yellowness) values being measured.

Bread colour is determined using the following equations:

$$\Delta L = L_{sample} - L_{control} \quad [8]$$

Where: +ΔL means the sample is lighter than the control

- ΔL means the sample is darker than the control

$$\Delta a = a_{sample} - a_{control} \quad [9]$$

Where: + Δa means the sample is more red than the control

- Δa means the sample is more green than the control

$$\Delta b = b_{sample} - b_{control} \quad [10]$$

Where: + Δb means the sample is more yellow than the control

- Δb means the sample is more blue than the control

Total colour change for the bread is calculated using Euclidean distance between the colours expressed as L, a and b:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \quad [11]$$

Parameter ΔE shows how much product deviate from the referent colour (Table 1).

Table 1. Significant differences between measured ΔE value and the referent value (Xiao, 2008).

ΔE	Meaning
0 – 0.5	Trace difference
0.5 – 1.5	Small difference
1.5 – 3.0	Noticable difference
3.0 – 6.0	Significant difference
6.0 – 12.0	Large difference
> 12.0	Very large difference

3.2.2.3. Crumb porosity

Bread loaves were cut in half and the coefficient of porosity of the crumb was determined using the Dallmann scale (Dallmann, 1958). The Dallmann scale ranges from 1 to 8, where 1 shows an extremely open heterogenous crumb structure with large and irregular cells, and 8 shows homogenous compact crumb structure with small and regular pores. The Dallmann scale is determined by visual observation and larger Dallmann scale values are more acceptable than small Dallmann scale values.

3.2.2.4. Texture Profile Analysis

Texture Profile Analysis is a double compression test used for determining textural properties of foods. During the test food samples are compressed twice using a texture analyzer which mimics the mouth's biting action. Compressing samples twice provides insight into how samples behave when chewed. The instrument begins recording data when the automatic trigger is achieved at the specific trigger force, after that the probe compresses the sample at the test speed and travels the target distance or percent strain. Once the target distance or strain is achieved the probe ascends to the original position at the test speed. Before the second compression occurs the instrument waits for the target time. Finally, the probe ascends all the way to the starting position at the post-test speed. Typical TPA parameters are hardness, fracturability, cohesiveness, springiness, gumminess, chewiness and resilience. Hardness value is the peak force that occurs during the first compression, cohesiveness is the area of work during the second compression divided by the area of work during the first compression and the gumminess is a result of hardness multiplied with cohesiveness. Springiness defines how well sample physically springs back after it has been deformed during the first compression and has been allowed to rest for the target wait time between the strokes, while the chewiness applies only to solid products and is calculated as guminess multiplied with springiness.

This test was carried out using ZWICK / ROELL Z010 test machine (Ulm, Germany) in 8 repetitions and it was consisted of a double compression of the samples with a flat plate. The degree of the sample compression was 40% and the relaxation time between two compressions was 30 seconds. The samples were prepared by cutting bread into cylinders with a height of 13 mm and a diameter of 17 mm.

3.2.3. Bread powder production

Bread loaves, after baking and after one day of storage, were cut into small cubes and frozen. Prior to chemical analysis the samples were thawed and milled using universal laboratory mill WZ-1 (ZBPP, Bydgoszcz, Poland).

3.2.4. Bread chemical analysis

3.2.4.1. Extraction procedure

For the extraction of phenolics was followed protocol similar to the one described by Lachowicz et al. (2017). Using analytical scale 2.0g of the bread powder samples were weighted and after that extracted with 10 mL of 80% methanol acidified with 1% HCl (v/v). The extraction was performed by incubation for 20 minutes under sonication (Sonic 6D, Polsonic, Warsaw, Poland) with occasional shaking. After 20 minutes the slurry was centrifuged at 19,000 x g for 10 minutes, and the supernatant was filtered through a Whatman no. 1 filter paper. Obtained supernatant was used for analysis.

3.2.4.2. Total polyphenol determination

A widespread method for determining phenolic compounds is by using Folin - Ciocalteu reagent. It is based on oxidation in alkaline solution of phenols with yellow molybdotungstophosphoric heteropolyanion reagent and is followed with colorimetric measurement of the resultant molybdotungstophosphate blue. Resulting blue pigments have a maximum absorption depending on the qualitative and/or quantitative composition of phenolic mixtures, as well as the pH of solutions, obtained by adding sodium carbonate (Cicco et al., 2008).

Total polyphenols were determined using the Folin-Ciocalteu method (Gao et al., 2000). An aliquot (100 μL) of extract was mixed with 2000 μL distilled water and 200 μL Folin-Ciocalteu phenol reagent. To the mixture was added 200 μL sodium carbonate solution (200 g L^{-1}) and the mixture was incubated at 20°C for 1 hour in the darkness. After incubation the absorbance was read at 765 nm on a UV-vis spectrophotometer (Shimadzu UV-2401 PC, Kyoto, Japan) and all the determinations were performed in triplicate. Gallic acid was used as a standard. Total polyphenolics were calculated using the following equation and then expressed as milligrams of gallic acid equivalents (GAE) per 100 g dry weight (DW).

$$y = 23.35 \times A - 1.675 \qquad [12]$$

Where is:

y – gallic acid concentration (mg L^{-1})

A – absorbance at 765 nm

3.2.4.3. Ferric reducing/antioxidant power (FRAP) assay

The total antioxidative potential of a sample was determined using a ferric reducing ability of plasma (FRAP) assay as a method for measuring antioxidant power. In this method ferric-tripyridyltriazine (Fe^{III}-TPTZ) complex, at low pH, is reduced to the ferous (Fe^{II}) form which causes formation of the ferrous-tripyridyltriazine complex. The reaction if followed by development of an intensive blue colour with an absorption maximum at 593 nm. FRAP values are obtained by comparing the apsorbance at 593 nm in the sample mixtures with the mixtures containing known concetration of the ferrous ions (Benzie & Strain, 1996).

FRAP reagent was prepared by mixing 300 mL acetate buffer (pH 3.6), a solution of 10 mg TPTZ in 40 mmol HCl, and 20 mg $FeCl_3$ at 10:1:1 (v/v/v). To the prepared reagent was added 10 mL of sample solution and mixed thoroughly. After 10 minutes the absorbance was measured at 593 nm using Shimadzu UV-2401 PC spectrophotometer (Kyoto, Japan). A standard curve was plotted using different concentrations of Trolox and all of the solutions were used on the day they were prepared. The results were calculated using the following formula and then corrected for dilution and expressed in μmol Trolox 100 g^{-1} dry weight (DW).

$$y = 94.13 \times A - 13.58 \qquad [13]$$

Where is:

y – Trolox concentration (μmol L^{-1})

A – absorbance at 593 nm

3.2.4.5. Free radical-scavenging ability determination with the use of a stable ABTS radical cation

ABTS radical cation decolourization assay is a method for determining antioxidant activity applicable to lipophilic and hydrophilic antioxidants, including flavonoids. Reaction of oxidation of ABTS with potassium persulfate results in formation of blue/green radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$). This compound has absorption maxima at wavelengths of 645 nm, 734 nm, 815 nm and the more commonly used maximum at 415 nm. Radical monocation ($ABTS^{\bullet+}$) is reduced to ABTS when the hydrogen-donating antioxidants are added. Extent of decolourization as percentage of inhibition of the $ABTS^{\bullet+}$ radical cation is a function of concentration and time, and it is calculated relative to the reactivity of Trolox as a standard, in the same condition.

The free radical-scavenging activity was determined using ABTS radical cation decolourization assay described by Re et al. (1999). ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS stock solution with 2.45 mL potassium persulfate (final concentration) and kept in the dark at the room temperature for 12-16 hours before use. When stored at the room temperature in the dark the radical was stable in this form for more than two days. The samples containing ABTS+ solution were diluted with redistilled water to the absorbance of 0.700 at 734 nm and equilibrated 30°C. To the 0.03 mL of polyphenolic extracts was added 3.0 mL of diluted ABTS+ solution and the absorbance was read exactly 6 minutes after the initial mixing. All determinations were performed in triplicate and using Shimadzu UV-2401 PC spectrophotometer (Kyoto, Japan). The results were calculated using the following formula and then corrected for dilution and expressed in μmol Trolox 100 g^{-1} dry weight.

$$\boldsymbol{y = -122.73 \times A + 85.491} \qquad \textbf{[14]}$$

Where is:

y – Trolox concentration (μmol L^{-1})

A – absorbance at 734 nm

3.2.5. Sensory evaluation

After the physical analysis bread was subjected to sensory analysis in well-lit laboratory. Before the analysis samples were prepared by cutting bread loaves in small cubes, labeled with random 3-digit code and served in random order. The questionnaire was based on subjective perception on external appearance, smell, texture, taste and overall liking. The sensory attributes were evaluated using hedonistic scale from 1 to 5, with 1 meaning the least liking, and 5 meaning the most liking.

3.3. STATISTICAL ANALYSIS

Obtained physical and chemical parameters of bread were analyzed using Statistica v.13.3.0 (StatSoft, Tulsa, USA). The data obtained for total polyphenol determination, ABTS and FRAP methods were treated by two-way ANOVA, while the data obtained for sensory analysis and TPA test were treated by one-way ANOVA. Post hoc tests are run to confirm where the overall statistically significant difference occurred between group means.

4. RESULTS AND DISCUSSION

In this work, the effect of addition of black garlic powder to the physical, chemical and sensory characteristics of bread has been investigated. Bread without black garlic powder addition was used as a control, while to the rest were added different concentrations of black garlic powder.

Physical characteristics of bread, determined during the experimental research, are volume, crumb porosity, colour, hardness, cohesiveness, springiness, gumminess, chewiness. The results for specific volume are shown in the Figure 3, the results for the crumb porosity are shown in the Table 2, the results for colour are shown in the Table 3, and the results for the TPA parameters (hardness, cohesiveness, springiness, gumminess and chewiness) are shown in the Table 4.

Chemical characteristics of bread were also observed. Total phenolic contents were determined using the Folin-Ciocalteu method and obtained results were shown in the Table 5. The total antioxidative potential was determined using a ferric reducing ability of plasma (FRAP) assay and the results were shown in the Table 6. The antioxidative activity was also determined using ABTS radical cation decolourisation assay, and the results were shown in the Table 7.

The sensory analysis was carried out in the research, and the results for the fresh bread were shown in Figure 6 and for the bread after 24 hours storage in Figure 7.

4.2. PHYSICAL CHARACTERISTICS OF BLACK GARLIC BREAD

4.2.1. Bread specific volume

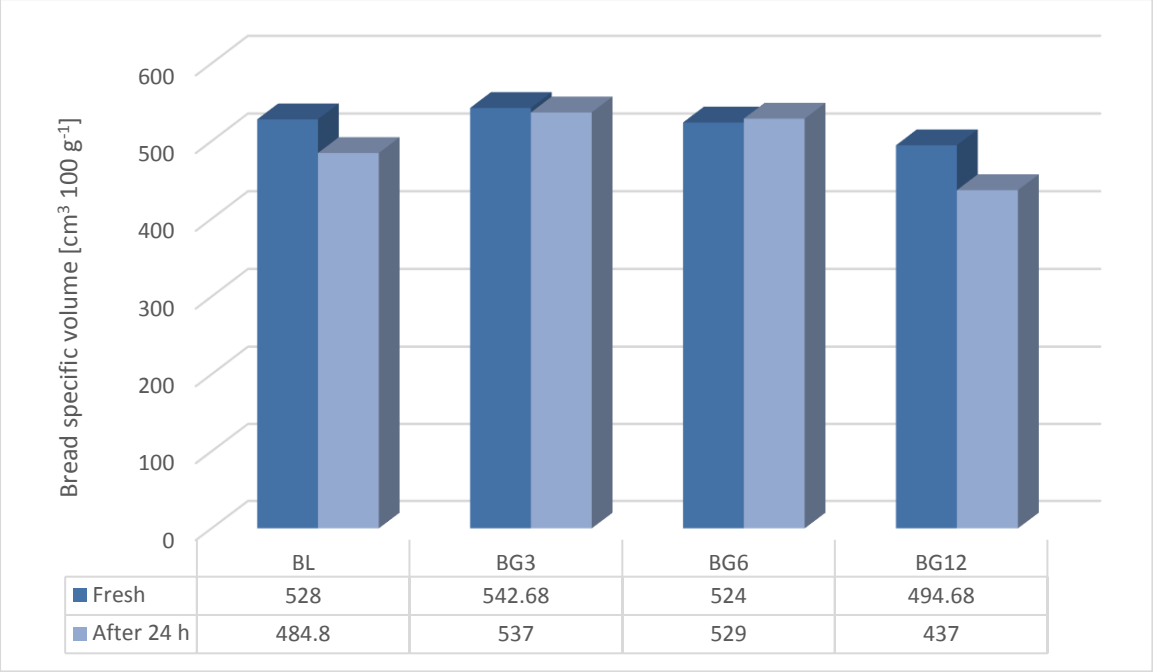

Figure 3. Bread specific volume of the fresh bread and bread after 24 hours (BL - control bread; BG3 - 3% added black garlic powder; BG6- 6% added black garlic powder and BG12- 12% added black garlic powder).

Breads with increased bread specific volume have better quality. This effect is due to the higher capacity to retain the gases which are formed during fermentation (Sciarini et al, 2012). Bread volume is one of the most important characteristics because it provides a quantitative measurement of baking performance (Tronsmo et al., 2003). It is also important for consumers because bread appear to be lighter and not so dense (Hathorn et al., 2008). In the Figure 3 are shown results of determining bread specific volume for fresh bread and bread after 24 hours, with black garlic powder addition of 0%, 3%, 6% and 12%. Bread specific volume for fresh bread is in the range from 494.68 cm^3 100 g^{-1} to 542.68 cm^3 g^{-1}, while the bread specific volume for the bread after 24 h storage is in the range from 437.00 cm^3 g^{-1} to 537.00 cm^3 g^{-1}. It can be seen that the bread specific volume for both fresh bread and bread after 24 h storage is the highest for the bread with 3% addition of black garlic powder (542.68 cm^3 g^{-1} and 537.00 cm^3 g^{-1}, respectively). From the results it can be concluded that black garlic powder addition has the effect on bread specific volume, addition of 3% caused the increase of the specific volume,

while the higher concentrations of black garlic powder caused the decrease of specific volume for the fresh bread. Storage also has an influence on bread specific volume, as it can be seen that the specific volume of the fresh bread is higher than the specific volume of the bread after 24 h storage. For the bread after 24 h storage, 3% black garlic powder addition caused smaller specific volume decrease compared to the control bread, 6% addition caused slight increase in the specific volume, while 12% addition caused bigger decrease of specific volume compared to the control bread. This is similar to the findings of Ju and other (2010) who reported that the higher addition of black garlic powder causes lower bread specific volume. Other studies have also reported higher specific volumes, e.g. 3.3-4.0 cm^3 g^{-1} for breads prepared with unheated maitake mushroom powder (Seguchi et al., 2001); 3.7-4.6 cm^3 g^{-1} for breads prepared with hydrocolloids (Bárcenas et al., 2004); and 3.4-4.4 cm^3 g^{-1} for wheat breads prepared with dextrins (Miyazaki et al., 2004).

4.2.2. Bread crumb porosity

Table 2. Results of determining bread crumb porosity

Sample	Crumb porosity
BL	5
BG3	6
BG6	7
BG12	7

Black garlic powder concentration 0%, 3%, 6% and 12% for the bread (BL, BG3, BG6 and BG12, respectively).

Observing the cross sections of bread loaves it was determined that the crumb porosity differ between the bread samples. Crumb porosity of the control bread was 5 according to the Dallmann scale. Bread with 3% addition of black garlic powder has Dallmann scale value of 6, while bread with 6% and 12% addition of black garlic powder have Dallmann scale value of 7. It can be concluded that black garlic powder addition has an effect on pore formation and the addition of 6% and 12% cause larger pore formation, and thus more acceptable crumb texture. The results can be compared to the findings of Wronkowska and other (2012) who observed the gluten-free bread with buckwheat flour addition have Dallmann scale value 4 for the control sample, value 7 for bread with 10% addition of buckwheat flour, and value 8 for

bread with 20-40% addition of buckwheat flour. According to Pejcz and other (2015) addition of non-cereal flours (chestnut, coconut, flax or hemp flour) did not affect crumb porosity.

Figure 4. Cross section of bread loaf with addition of 0%, 3%, 6% and 12% of black garlic powder, respectively.

4.2.3. Bread colour

Table 3. Results of determining bread colour

Sample	Colour							
	Fresh				After 24 h			
	ΔL*	Δa*	Δb*	ΔE	ΔL*	Δa*	Δb*	ΔE
BG3	-34.30	9.25	-10.72	37.11	-31.58	8.79	-9.13	34.03
BG6	-39.34	8.26	-16.13	43.31	-36.36	7.95	-14.79	40.05
BG12	-42.61	6.70	-20.06	47.57	-40.08	6.21	-19.37	44.95

Black garlic powder concentration 3%, 6% and 12% for the fresh bread and bread after 24 hours (BG3, BG6 and BG12, respectively).

Important bread characteristic is colour and consumers use colour, in terms of darkness or lightness, when selecting bread (Hathorn et al., 2008). The results can be seen in the Table 4. Lower L* value means the product has darker colour, while higher L* value means the product has lighter colour. From the results it can be seen that the darkest bread is the one made with 12% addition of black garlic and the lightest bread is the one made without addition of black garlic powder. This is observed for both fresh bread and bread after 24 hours, but it can also be seen that the bread after 24 hours storage is lighter than the fresh bread. a* value shows amount of red or green component and from the results it can be observed that bread with black garlic powder is more red than the control sample, and bread with 3% addition of black garlic powder is the one that is the most red ($\Delta a^* = 9.25$). Bread after storage appears to be less red than the fresh bread. b* value shows amount of blue or yellow component and from the results can be seen that the control bread is the most yellow, followed by bread with 3% addition of black garlic, while the least yellow bread is the one with 12% addition of black garlic. Fresh bread appears to be less yellow than the bread after 24 hours storage. ΔE value shows how much the sample differs from the control sample. Results show that all bread loaves with black garlic powder addition differ significantly (>12) from the control sample, bread without black garlic powder addition. Bread after 24 h storage show less difference from the control sample than the fresh bread, but the difference is still significant.

Figure 5. Wheat bread with addition of 0%, 3%, 6% and 12% black garlic powder, respectively.

4.2.4. Texture Profile Analysis

Table 4. The effect of black garlic powder addition to the wheat flour bread on the TPA parameters

TPA parameters	Control 0 h	3% BG 0 h	6% BG 0 h	12% BG 0 h	Control 24h	3% BG 24 h	6% BG 24 h	12% BG 24h
Hardness [g]	1.65cA ± 0.51	1.59aA ± 0.65	1.63bA ± 0.36	2.39dB ± 0.45	3.45cB ± 0.83	2.17aA ± 0.58	2.57bA ± 0.48	3.77dB ± 0.28
Cohesiveness [%]	0.86dC ± 0.01	0.86cBC ± 0.01	0.85bAB ± 0.01	0.83aA ± 0.01	0.78bAB ± 0.02	0.78cA ± 0.02	0.78dA ± 0.01	0.76aB ± 0.01
Gumminess	1.43cAB ± 0.43	1.36aA ± 0.54	1.38bA ± 0.30	1.99dB ± 0.35	2.68cB ± 0.60	1.69aA ± 0.41	2.01bA ± 0.38	2.85dB ± 0.22
Springiness [%]	0.96bA ± 0.01	0.96dA ± 0.01	0.96aA ± 0.01	0.96cA ± 0.01	0.97dA ± 0.003	0.96bAB ± 0.005	0.97cA ± 0.004	0.96aB ± 0.012
Chewiness	1.36cAB ± 0.42	1.31aA ± 0.53	1.32bA ± 0.29	1.91dB ± 0.34	2.60cB ± 0.58	1.62aA ± 0.40	1.95bA ± 0.37	2.72dB ± 0.22

Black garlic powder concentration 0, 3, 6 and 12 % w/w for fresh bread and for bread after 24 h (Control 0h, 3 % BG 0h, 6 % BG 0h12 % BG 0h, Control 24h, 3% BG 24h, 6% BG 24h and 12% BG 24h, respectively). Data expressed as mean ± standard deviation. Values followed by the same letter in the same row (a, b, c, d) or column (A, B, C) are not significantly different at 95 % confidence level for the given TPA parameter after 0 hours or after 24 hours.

Texture Profile Analysis (TPA) revealed that the bread with 3% addition of black garlic powder has the lowest hardness, followed by bread with 6% black garlic powder addition. Control bread shows small difference from the bread with 6% black garlic addition, while the bread with 12% black powder addition appears to have the highest hardness. This is the same for both fresh bread and bread after 24 h storage, with control sample (bread without black garlic powder addition) having significant difference in hardness value. It can be concluded that black garlic powder has an influence on bread hardness, causing bread to appear softer after storage. Storage also has influence on bread hardness, causing bread to be harder after 24 h storage. These results can be compared to the results from the study that Wang and other (2002) conducted, they concluded that carob fibre or pea fibre supplementation on the wheat bread caused the decrease of crumb hardness, while inulin addition caused the increase of crumb hardness. Other studies have found that wheat bread with barley addition has increased hardness compared to the control (Sullivan et al, 2011). Ulziijargal and other (2013) also observed the influence that storage has on bread hardness. They have found that after 2 to 6 days of storage white bread became significantly harder than mycelium-supplemented bread.

Influence of black garlic powder on cohesiveness can be seen from the results. Bread with 12% black garlic powder has the lowest cohesiveness, followed by bread with 6% black garlic powder, while the control bread has the highest cohesiveness. After 24 hours storage all bread samples have lower cohesiveness values, with bread with 12% black garlic powder having the lowest cohesiveness value compared to other samples.

Bread with 12% black garlic powder has the highest gumminess value, followed by control bread. As for bread after 24 hours storage, gumminess values have significantly increased, with bread with 12% black garlic powder addition has the bread with the highest gumminess value, followed by control bread. Bread with 3% black garlic powder addition has the lowest gumminess value, for both fresh bread and bread after 24 hours storage.

From the results can be seen that the black garlic powder does not have influence on bread springiness. As for fresh bread, springiness value is the same for control bread and bread with black garlic powder addition (0.96% ± 0.01). Springiness value for control bread after 24 hours is the same as for the bread with 6% black garlic powder (0.97% ± 0.01). Bread samples with 3% and 12% black garlic powder additon have slight decrease in springiness value, which is the same as for the fresh bread (0.96% ± 0.01).

Bread with 3% black garlic powder has the lowest chewiness value (1.31 ± 0.53), followed by bread with 6% black garlic powder (1.32 ± 0.29), and bread with 12% black garlic powder having the highest chewiness value (1.91 ± 0.34). Storage time has an influence on chewiness, especially on control bread and bread with 12% black garlic powder, with chewiness values of 2.60 ± 0.58 and 2.72 ± 0.22, respectively.

4.3. CHEMICAL CHARACTERISTICS OF BLACK GARLIC BREAD

4.3.1. Total phenolic content

Table 5. The effect of black garlic powder addition to the wheat flour bread on the total phenolic content determined with Folin-Ciocalteu method

		Control	3% BG	6 %BG	12% BG
Total phenolic content [mg 100 Gallic acid g^{-1} dry mass]					
Time [h]	0	30.04bA ± 0.40	74.52dB ± 2.22	113.78fE ± 0.23	166.10hC ± 7.10
	24	26.96aA ± 1.17	66.42cB ± 1.29	99.07eD ± 0.62	161.04gC ± 7.78

Black garlic powder concentration 0, 3, 6 and 12 % w/w (Control, 3 % BG, 6 % BG and 12 % BG, respectively). Data expressed as mean ± standard deviation. Values followed by the same letter in the same row (a, b, c, d, e, f, g, h) or column (A, B, C, D, E) are not significantly different at 95 % confidence level.

Total phenolic content in the bread was determined using Folin-Ciocalteu method. Several components affect the detected antioxidant composition or capacity, including the intrinsic phenolic compounds of flour, from ingredients naturally containing phenolics, intermediate phenolic compounds generated during baking (Michalska et al., 2008), thermal-induced degradative products (Rupasinghe et al., 2008) and/or polyphenol-polysaccharides complexes (Shahidi & Naczk, 1995). Total phenolic content in whole black garlic bulbs range from 38.6 ± 0.45 to 163.2 ± 0.14 mg Gallic acid 100 g^{-1}, and in peeled black garlic from 31.1

± 0.08 to 187.7 ± 0.30 mg Gallic acid 100 g^{-1}, depending on length of heat treatment and processing temperature (Toledano-Medina et al., 2016). Due to black garlic being rich in polyphenols, results show that bread 12% black garlic addition contains the biggest total phenolic content, while the control bread has the lowest total phenolic content. These results can be compared to the results from other studies. Ajila and others (2007) observed that the addition of mango peel powder to the biscuits caused the increase in the phenolic content. On the other hand, Bilgiçli and others (2007) observed that the addition of apple or lemon fibre did not change the total phenolic content. Storage time has an influence on phenolic content, causing the decrease in total phenolic content compared to the fresh bread. This can be seen from the results, bread after 24 hours storage has the lower phenolic content than the fresh bread.

4.3.2. Ferric reducing/antioxidant power (FRAP) assay

Table 6. The effect of black garlic powder addition to the wheat flour bread on the antioxidant capacity determined with FRAP assay

		Control	**3% BG**	**6 %BG**	**12% BG**
FRAP [µmol Trolox 100 g^{-1} dry mass]					
Time [h]	0	74.39bB ± 3.66	107.03dD ± 0.98	197.70fF ± 3.83	249.95hH ± 0.98
	24	63.57aA ± 3.59	94.16cC ± 1.18	178.25eE ± 3.09	236.30gG ± 0.27

Black garlic powder concentration 0, 3, 6 and 12 % w/w (Control, 3 % BG, 6 % BG and 12 % BG, respectively). Data expressed as mean ± standard deviation. Values followed by the same letter in the same row (a, b, c, d, e, f, g, h) or column (A, B, C, D, E, F, G, H) are not significantly different at 95 % confidence level.

Antioxidant capacity of black garlic bread was determined using FRAP method which is based on reduction of ferric-tripyridyltriazine (Fe^{III}-TPTZ) complex, at low pH, to the ferous (Fe^{II}) form which causes formation of the ferrous-tripyridyltriazine complex. The results are shown in the Table 6.

The largest antioxidant capacity shows bread with 12% addition of black garlic powder, for both fresh bread and bread after 24 hours storage (249.95 μmol Trolox 100 g^{-1} and 263.30 μmol Trolox 100 g^{-1}, respectively). Lowest antioxidant capacity shows bread without black garlic powder addition, for both fresh bread and bread after 24 h storage (74.39 μmol Trolox 100 g^{-1} and 63.57 μmol Trolox 100 g^{-1}, respectively). This is due to black garlic being rich in antioxidants and therefore bread with higher concentration of black garlic powder shows higher antioxidant capacity. Obtained results can be compared to the results obtained by Borczak and others (2016). In their study was observed that all breads enriched with fruits show higher antioxidant capacity than the control bread. Antioxidant capacity determined using FRAP method shows the same trend as total phenolic content, antioxidant capacity is larger in the fresh bread than in the bread after 24 h storage. These results are in contrast to the study that Holtekjølen and others (2008) conducted, they have observed that storage did not have significant influence on antioxidant properties on bread with the addition of barley flour.

4.3.3. Free radical-scavenging ability determination with the use of a stable ABTS radical cation

Table 7. The effect of black garlic powder addition to the wheat flour bread on the antioxidant capacity determined with ABTS assay

		Control	**3% BG**	**6 %BG**	**12% BG**
ABTS [μmol Trolox 100 g^{-1} dry mass]					
Time [h]	0	10.22bAB ± 1.02	28.39dC ± 2.55	59.00fD ± 2.19	147.22gF ± 10.18350
	24	7.44aA ± 2.55	22.89cBC ± 3.64	55.33eD ± 3.84	155.83hF ± 10.61

Black garlic powder concentration 0, 3, 6 and 12 % w/w (Control, 3 % BG, 6 % BG and 12 % BG, respectively). Data expressed as mean ± standard deviation. Values followed by the same letter in the same row (a, b, c, d, e, f, g, h) or column (A, B, C, D, E) are not significantly different at 95 % confidence level.

Antioxidant capacity of black garlic bread was also determined using ABTS method, based on reaction of oxidation of ABTS with potassium persulfate where blue/green radical monocation $ABTS^{\bullet+}$ is formed and then reduced to ABTS when the hydrogen-donating antioxidants are added. The results are shown in the Table 8. The largest antioxidant capacity shows bread with 12% addition of black garlic powder, for both fresh bread and bread after 24 h storage (147.22 μmol Trolox 100 g^{-1} and 155.83 μmol Trolox 100 g^{-1}, respectively). Lowest antioxidant capacity shows bread without added black garlic powder (10.22 μmol Trolox 100 g^{-1} and 7.44 μmol Trolox 100 g^{-1}, respectively). As mentioned before, this is due to black garlic being rich in polyphenols and because of that black garlic bread with higher concentrations of black garlic powder has higher antioxidant activity. Previous studies report that replacing wheat flour with ground flaxseed up to 8% (w/w) increases antioxidant activity, determined with ABTS method (Meral & Sait Dogan, 2013). Changes occuring after 24 h storage are not significant compared to the fresh bread ($p>0.05$) which is the same for the results that Holtekjølen and others (2008) obtained from the study.

4.4. SENSORY ANALYSIS

A sensory evaluation was conducted on the black garlic breads to study the sensory profile of each type of bread. The sensory evaluation showed sensory differences between the breads baked with the different black garlic powder concentrations compared to the control, for both fresh bread (Fig. 5) and bread after 24 hours storage (Fig. 6). Evaluated attributes were external appearance, smell, structure, taste and general liking. Sensory attributes were evaluated using the hedonistic scale from 1 to 5 (1 – the least liking, 5 – the most liking). The scores are shown in the spider diagram as mean values for each sensory attribute for fresh bread (Fig. 6) and for bread after 24 hours storage (Fig. 7). The higher the score in the spider diagram, the more intense is this attribute of the breads.

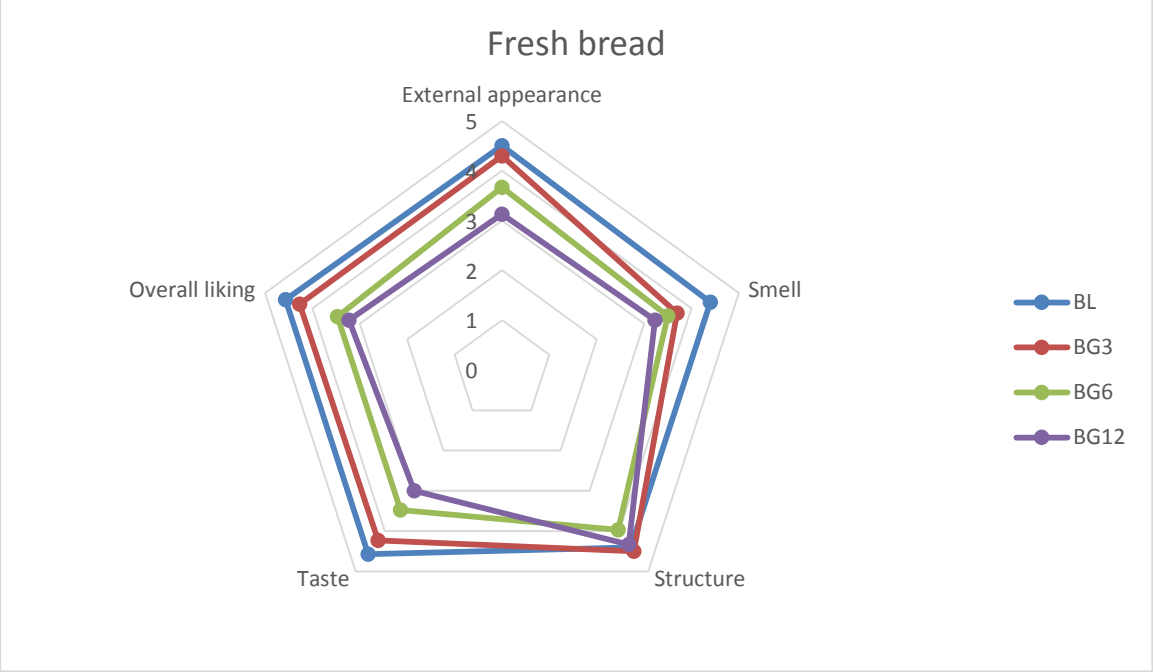

Figure 6. The effect of black garlic powder addition to the wheat flour bread on the sensory parameters after baking

Results for the external appearance of the fresh bread show that the control bread (BL) achieved highest score (4.50), while bread with 12% black garlic powder addition achieved the lowest score (3.13). As for the smell, control bread again achieved the highest score (4.40) and bread with 12% black garlic powder addition achieved the lowest score (3.23). Results for the structure show that bread with 3% black garlic powder addition achieved the highest score (4.50) and bread with 6% black garlic powder addition achieved the lowest score (3.97). The highest score in evaluation of the taste achieved control bread (4.57), while bread with 12% black garlic powder addition achieved the lowest score (3.00). The most accepted bread in terms of overall liking was control bread (4.57), while bread with 12% black garlic powder addition was the least accepted (3.23).

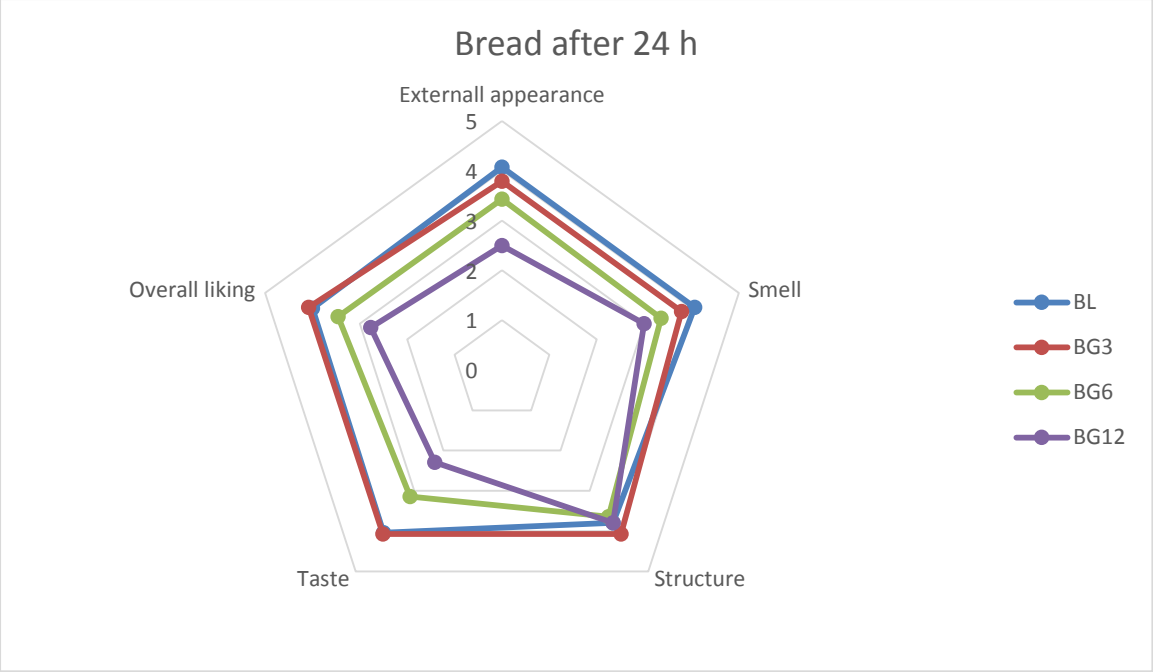

Figure 7. The effect of black garlic powder addition to the wheat flour bread on the sensory parameters after 24 h storage

Results for the external appearance for the bread after 24 hours storage show that the most accepted bread is control bread (4.07), while bread with 12% black garlic powder addition is the least accepted (2.50). Results for the smell show that control bread achieved the highest score (4.07) and bread with 12% black garlic powder addition achieved the lowest score (3.00). The highest score in evaluation of the structure achieved bread with 3% black garlic powder addition (4.07) and bread with 6% black garlic powder addition achieved the lowest score (3.64). As for the taste, bread with 3% black garlic powder addition achieved slight higher score (4.07) than the control bread (4.04), while bread with 12% black garlic powder addition achieved the lowest score (2.29). The most accepted bread in terms of overall liking was bread with 3% black garlic powder addition (4.08), while bread with 12% black garlic powder addition was the least accepted (2.77).

From the results can be seen that storage has negative influence on sensory acceptance of the bread, as the bread after 24 hours storage achieved lower scores for all the evaluated attributes. It can also be seen that 3% black garlic powder addition has positive influence on bread structure, for both fresh bread and bread after 24 hours storage. Black garlic powder addition in 3% concentration for bread after 24 hours storage achieved better scores in taste and overall liking compared to control bread, indicating that 3% addition of black garlic powder could be used to preserve good sensory characteristics of the bread after the storage.

5. CONCLUSIONS

Based on the presented results and the discussion, the following conclusions are reached:

1. Addition of 3% of black garlic powder caused the increase of bread specific volume, further addition caused the decreased of bread specific volume. After 24 hours storage, bread with 3% black garlic powder addition had smaller decrease in specific volume, compared to the control bread.
2. Black garlic powder has an influence on bread crumb porosity. Higher addition of the black powder cause larger pore formation, and thus more acceptable crumb structure.
3. Storage time does not have significant influence on bread colour
4. Bread with 3% black garlic powder addition shows the best scores for almost all TPA parameters (hardness, cohesiveness, gumminess, springiness, chewiness). Storage time has the biggest influence on hardness, and only slight influence on cohesiveness and springiness.
5. Total phenolic content of breads increase with the increase of black garlic powder addition. Storage time caused loss of phenolic content.
6. Antioxidant capacity of breads increase with the increase of black garlic powder addition. Storage time caused decrease of antioxidant capacity.
7. All attributes (external appearance, smell, structure, taste, overall liking) of bread with 3% black garlic powder addition, both fresh and after 24 hours storage, achieved high scores. This shows good sensory characteristics and high acceptance from consumers.

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STATEMENT OF ORIGINALITY

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

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