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The Effect of Black Cumin Seed Oil on The Oxidation Process of Lipids In Cookies.

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ABSTRACT

It has been studied the effect of black cumin seed oil on the stability of fat phase of flour confectionery products during storage. The effect of black cumin seed oil on the oxidation process of lipids was evaluated by several methods during the storage period: by restoration of potassium ferricyanide, by reaction with 2,2-diphenyl-1-pikrilgidrazil and by change in the peroxide value. It is found that the ethanolic extracts from the products with the addition of black cumin seed oil have had higher antioxidant and antiradical activity compared with the control samples. During the whole storage period, the control and test samples were observed a decrease in the antioxidant status, but the antioxidant activity of the products with the black cumin seed oil remained at a higher level compared with the samples without it. It is demonstrated that the products that have not contained the black cumin seed oil resulted in the sevenfold increase in the peroxide value, and after 60 days of storage its value was 13.4 mmol 1/2 O/kg; the addition of black cumin seed oil resulted in the fact that this figure has only doubled and amounted to 4.3 mmol 1/2 O/kg in the products that have not contained the black cumin seed oil. Thus, the addition of black cumin seed oil to the cookie formulation has a protective effect on its fat phase, protecting it from oxidation.

Keywords: *Nigella sativa,* black cumin seed oil, antioxidant activity, antiradical activity, lipid oxidation.

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INTRODUCTION

The actual problem of food production is to keep food quality and increase their shelf life. The flour confectionery products are popular and daily food product among the population. However, due to the presence of large amounts of fats in their structure, they are insufficiently proof upon their storage. During storage the fat-containing products accumulate the products of lipid oxidation: peroxides, hydroperoxides and secondary derivatives, which give rise to the unpleasant flavor and reduced nutritional value of the products. These compounds can also be potentially dangerous to human health, since the action of free radicals on the body leads to the appearance of various chronic diseases, decreased immunity and premature aging of the body [1].

The synthetic and natural antioxidants are used to increase proof of the food products containing fats. The synthetic antioxidants, such as tret-butylhydroquinone E 319 and butylhydroxyanisole E 320, are widely used in the food industry. Despite the fact that they are relatively cheap and slow down the process of fat oxidation in the food products, their use may cause a negative reaction from the consumers. In this regard, it is observed the worldwide trend of increasing the proportion of natural antioxidants in the food products. The natural antioxidants are considered to be more effective and safe, and can have a positive impact on the human health [2].

It is known that a lot of herbs, spices, oils and extracts have the ability to slow down the process of fat oxidation. One of these spices is the black cumin seeds. Black cumin (*Nigella sativa* L.) (black-caraway, kalonji) - an annual herbaceous plant of the ranunculaceous family (*Ranunculaceae*), native to Asia and the Mediterranean.

Since ancient times, the black cumin has been widely used in cooking and alternative medicine. It was highly valued in cooking for its pleasant taste and aromatic properties, as well as for the preservative action that helped to maintain the quality of food products during their processing and storage [3, 4].

Currently, the black cumin is widely used in food, cosmetic and pharmaceutical industries. The black cumin has antiasthmatic, anti-tumor, anti-viral, anti-bacterial, anti-inflammatory, anti-diabetic, anti-atherosclerotic and anti-oxidant effect [5].

The black cumin seed oil contains a large amount of active ingredients, such as saturated and unsaturated fatty acids, lipase, alkaloids, catechins, acetylcholines and others. The most active components include: thymoquinone, thymohydroquinone, dythymoquinone, *p*-cymene, carvacrol, 4-terpineol [4]. These compounds can inhibit oxidative processes in the lipid phase during the storage of flour confectionery products.

The objective of this study was to increase the shelf life of flour confectionery products (cookies) by introducing a natural antioxidant into its formulation - the black cumin seed oil.

MATERIALS AND METHODS

This paper was devoted to the study of the effect of essential oil of black cumin (Baraka, "BioExtracts (PVT) Ltd", Sri Lanka) as an additive in the flour confectionery products (cookies).

Ingredients of the flour product (per 1000 g of finished product): wheat flour, high-grade - 495.0 g; butter - 230.0 g; sugar - 155.0 g; chicken eggs - 125.0 g; sodium bicarbonate - 1.1 g (control). The test samples were added with the black cumin seed oil at a concentration of 1% (12.0 g).

The samples were prepared at a temperature of 200-210 °C for 10 min. The storage was carried out in a dark place at room temperature and at a relative humidity below 75%. The analysis of samples was carried out after 30 and 60 days.

A sample preparation for the determination of antioxidant and antiradical properties was carried out as follows: we placed 5 g of the ground sample in a beaker of 100 ml, then we added 50 ml of 70% ethanol, heated to a temperature of 70 °C, and were intermixing them for 10 minutes on a magnetic stirrer with

heating. The obtained extract was filtered through the filter "white ribbon", and after cooling to room temperature we brought it up to the initial volume of 50 ml by using 70% ethanol.

A determination of antioxidant properties of the finished product extracts was performed by the ferricyanide method according to Lertittikul W. et al. [6]. An optical density of the reaction mixture was measured at 700 nm on a spectrophotometer SF-2000 (Russia). The antioxidant activity of 1 g of sample was expressed as a percentage of 0.01% solution of ascorbic acid, which reducing capacity was taken as 100%.

The antiradical activity was determined by the method proposed by Brand-Williams W. et al. to reduce the optical density of the solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany) in the presence of antioxidants [7]. An optical density of the reaction mixture was measured at 517 nm on a spectrophotometer SF-2000 (Russia). A percentage of inhibition of DPPH radicals was determined by the formula:

$$\% \text{ of inhibition of DPPH} = [1 - (D_O/D_K)] \times 100,$$

where D_O – the optical density of test sample;
D_K – the optical density in the absence of antioxidants (control).

Simultaneously, we determined the percentage of inhibition of DPPH for the well-known concentrations of water-soluble analogue of Vitamin E - (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid Trolox (Sigma-Aldrich, Germany) and built a calibration curve.

The antiradical activity of the samples was expressed in mmol/g Trolox-Equivalent - the standard units, corresponding to the activity of a known concentration of synthetic antioxidant Trolox.

The processes of oxidative damage to the samples were evaluated by the change in peroxide value of lipid fraction. The lipid fraction of the finished products was extracted from the samples by extraction with hexane mixture of: isopropanol in the ratio (3:2) by the method proposed by Hara A. and Radin S. N. [8]. The weighted portion of cookies of 10 g was crushed and extracted with 50 ml of hexane mixture of: isopropanol for 10 minutes on a magnetic stirrer. The obtained extract was filtered in the pre-weighed flasks, and then the solvent was distilled off under vacuum at 70 °C. After distilling off the solvent, the flasks were dried in a drying cabinet at a temperature of 70 °C to constant weight and the mass of released fat was determined by the weight difference of the flasks. The obtained lipid fractions were used to determine the peroxide value under ISO 3960:2007.

RESULTS

It is known that many spice plants contain the antioxidant compounds in their composition, but many of them are unstable to heating. In our experiments, we investigated the antioxidant capacity of black cumin seed oil after heat treatment in the production of flour confectionery products, as well as its effect as a food additive, inhibiting the oxidative processes in the fat phase during storage.

With this purpose, we added the black cumin seed oil in the confectionery products (cookies) at a concentration of 1% and investigated the change in antioxidant and antiradical properties of the samples in the dynamics of the storage process.

The research results showed that the antioxidant activity determined under the reducing capacity of cookie extracts was reducing during storage, both in the test and control samples (Fig. 1). If immediately after the heat treatment (baking) the reducing force of 1 g of test sample was 9.8% relative to the ascorbic acid, and of control sample - 9%, then after 60 days of storage these values of the test and control samples were equal to 8.7 and 6.3%. In general, the reducing capacity of the products with the addition of black cumin seed oil was more pronounced during the storage period. Presence of a higher reducing activity in the test sample indicates the introduction of additional compounds by the black cumin seed oil interacting with the oxidants and the preservation of their activity after heat treatment.

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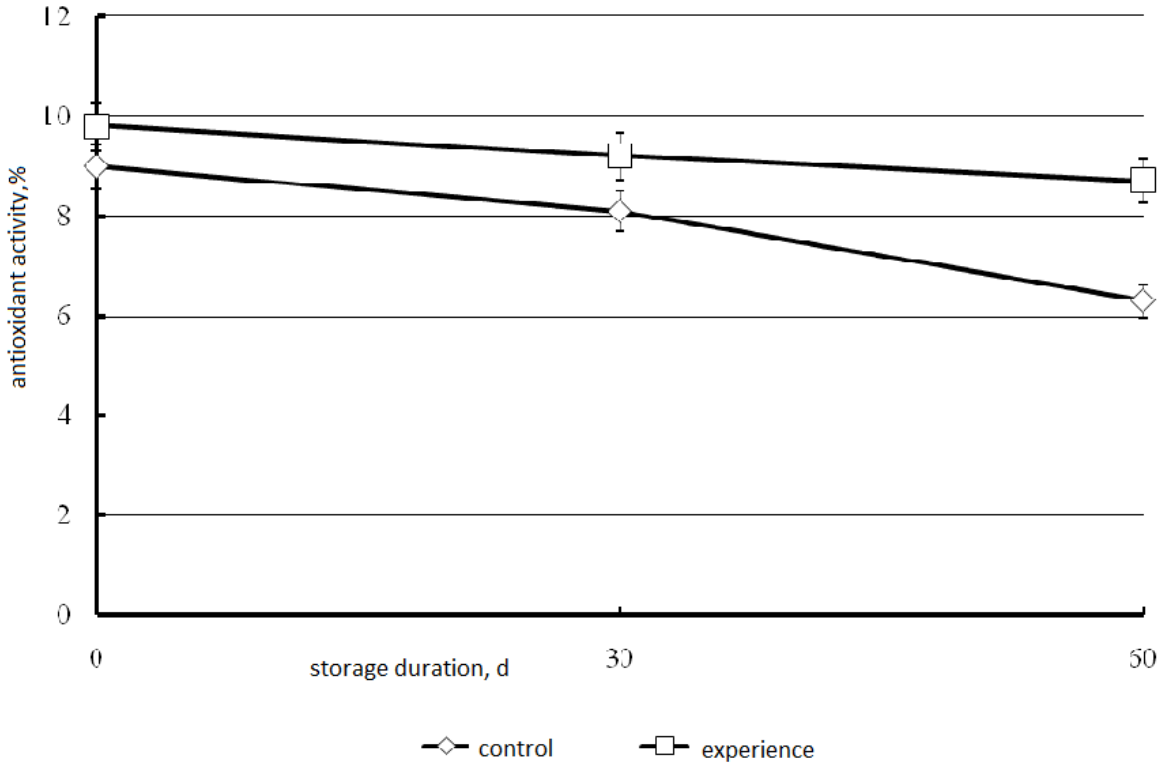

Figure 1 - Change in the antioxidant activity of cookies during storage

According to some researchers, the high antioxidant activity of black cumin seed oil is explained by the presence in its composition of a large number of thymoquinone - the biologically active compound, having a broad spectrum of therapeutic actions: antioxidant, anti-inflammatory, antihistamine, immunomodulating and anti-tumor [4, 9].

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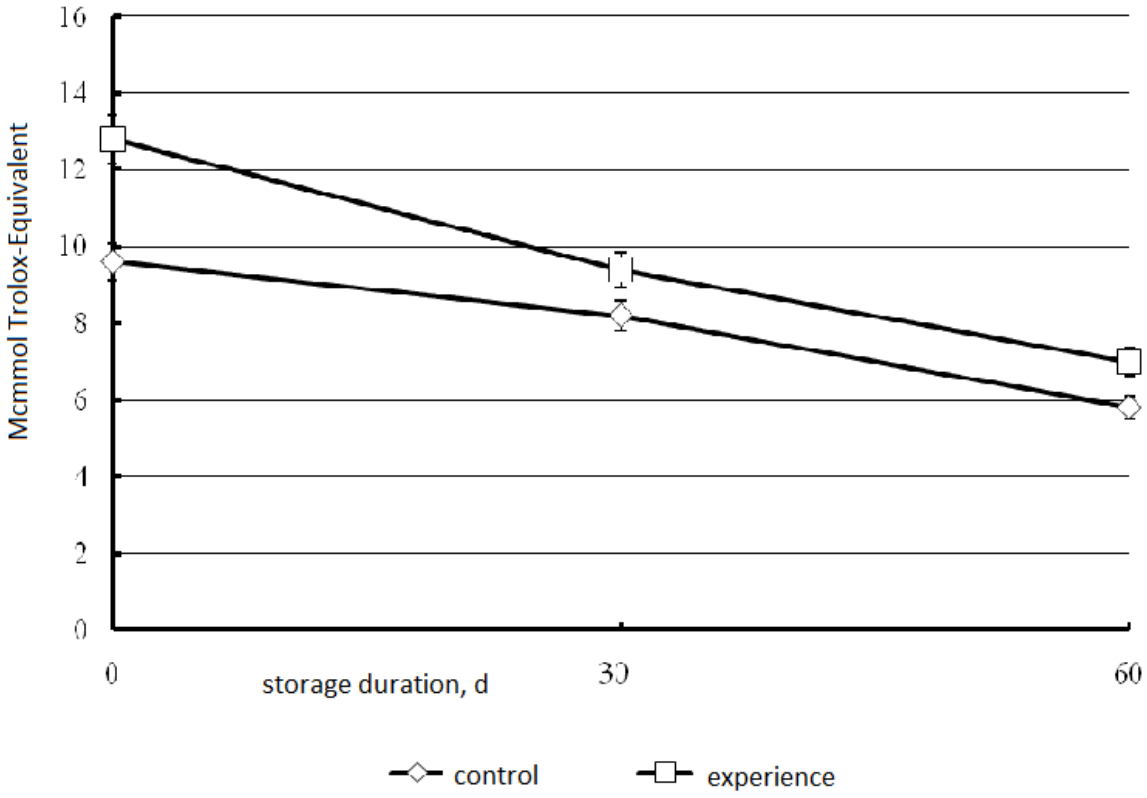

Figure 2 - Change in the antiradical activity of cookies during storage

The free radicals belong to the most reactive forms of oxidants. The formation of such compounds in the food products is capable to lead quickly to the product spoilage. To bind the free radicals there are used the substances capable to donate easily the electrons - antioxidants, which are abundantly present in spices and their extracts and oils.

The determination of antiradical properties of cookies with the black cumin seed oil after baking showed that these values were significantly higher (1.3 times) than the control sample and amounted to 12.8 mmol/g Trolox-Equivalent and 9.6 mmol/g Trolox- Equivalent, respectively (Fig. 2).

The higher antiradical activity of the test sample is apparently due to the antioxidant compounds contained in the black cumin seed oil. During storage, the difference between the level of anti-radical activity in the control and test samples was reduced, but remained significantly distinguishable. Probably the antiradical activity demonstrated by the test samples is due to a large number of pharmacologically active quinones (thymoquinone, dythymoquinone, thymohydroquinone, thymol, carvacrol), contained in the black cumin seed oil. [4] It was also reported that the antiradical properties of the black cumin extracts were proportionally increased with the increase of phenolic compounds in their content [10]. The substances of a similar nature found in the black cumin include: *p*-coumaric, hydroxybenzoic and syringic acids [10].

During the process of oxidative spoilage of the products, their fat phase accumulates the primary peroxide compounds, which in turn are also strong oxidants, and trigger the avalanche oxidation reaction chain with the formation of the secondary more stable compounds, such as alcohols, aldehydes, ketones and acids giving unpleasant taste and smell to the food products. This leads to a decrease in the nutritional value of the food products and deteriorates their stability during storage.

The degree of oxidation of fat phase of cookies was evaluated under the indicator "peroxide value". The indicator "peroxide value" determines the content of peroxides and hydroperoxides, and is therefore an important indicator of oxidative spoilage (Fig. 3).

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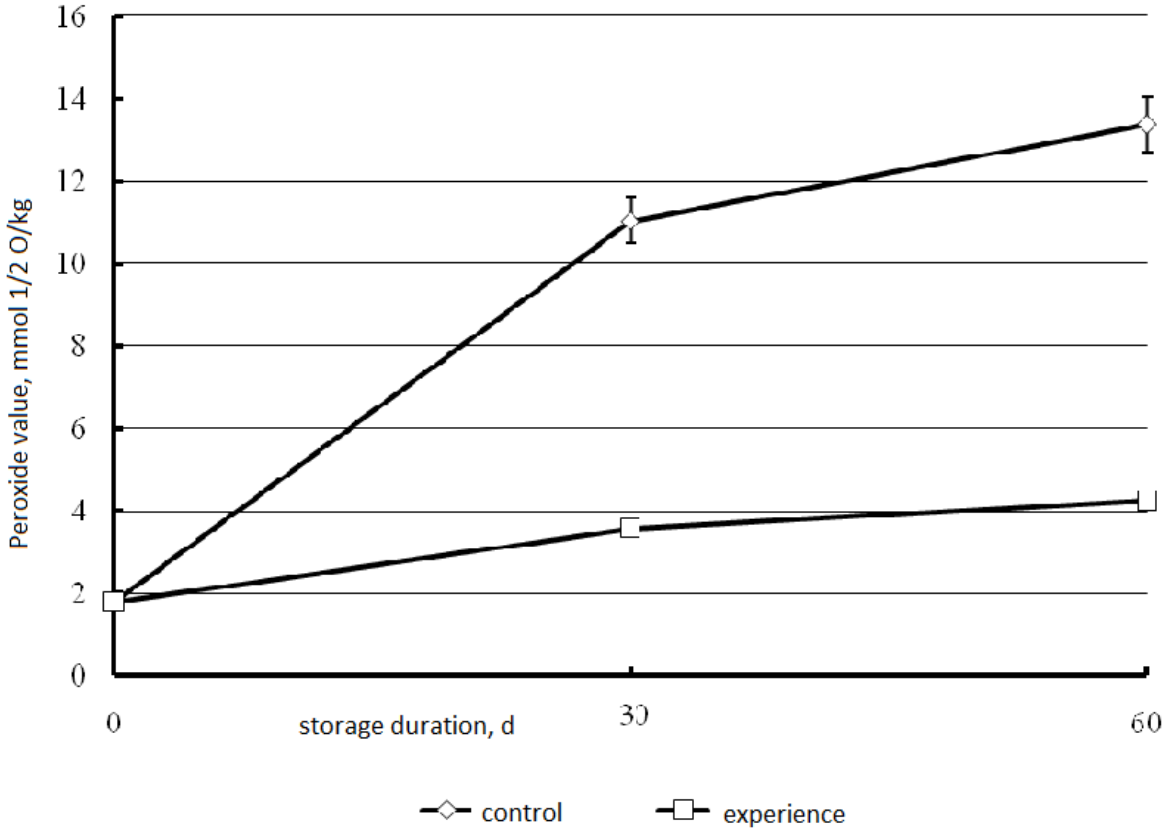

Figure 3 - Change in the peroxide value of lipid phase of cookies during storage

According to the obtained results, the indicators of peroxide value of the lipid fraction of the test and control samples were the same and amounted to 1.8 mmol 1/2 O/kg immediately after sample baking. Upon storage of cookies, this indicator increased with varying intensity in the test and control samples. There was observed that the cookies with the black cumin seed oil included the inhibition of accumulation of peroxides, while their number in control samples increased significantly. After 60 days of storage the peroxide content in the control sample was 13.4 mmol/kg 1/2 O/kg, and in the test samples - 4.3 mmol/kg 1/2 O/kg. Thus, by the end of the probationary period of storage the indicator of peroxide value of lipid control fraction was almost three times more than the test sample of cookies with the black cumin seed oil.

A significant impact on the rate of increase of peroxide value is made by the nature of active ingredients *Nigella sativa.* It was so shown that the minimum value of peroxide value in corn oil kept at 70 °C for 72 hours was in the presence of methanolic extract of the black cumin, and the maximum - in the presence of aqueous extract [10]. Thus, the low polar compounds of black cumin have the most efficient inhibition effect on the formation of peroxides, so it is advisable to use the oil of *Nigella sativa* as a protective agent for the lipid fraction of cookies.

The addition of black cumin seed oil also significantly influenced the organoleptic characteristics of finished products. The taste and flavor of products were undergone the biggest changes. The samples with the oil of *Nigella sativa* had a pleasant aroma and taste peculiar to this spice.

SUMMARY

The conducted studies have shown that the use of black cumin seed oil in the flour confectionery technology slows down the oxidation process of lipid fraction of the finished products, thereby increasing its shelf life. Furthermore, the addition of black cumin seed oil can improve the nutritional value of cookies by

increasing a content of the biologically active substances in them, as well as expand the range of flour confectionery products with the original taste and aromatic properties.

CONCLUSIONS

The black cumin seed oil can be recommended for increasing the resistance of fat-containing flour confectionery products to oxidative damage. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

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