



THE POSSIBILITIES OF INCREASING LIGNAN CONTENT IN FOOD

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ABSTRACT

Lignans are bioactive substances which belong to polyphenols. These compounds can be found in plants including coniferous trees. Lignans are secondary plant metabolites with a wide range of biological effects, such as antimicrobial, antiviral or anticancer. They also serve as antioxidants and are naturally occurring compounds which are found in food rich in fibre. There are more than 200 lignans that originate from more than 70 plant families. They can be found in all parts of the plant, mainly in seeds. Almost 37% of total lignan intake in human diet comes from drinking tea and coffee. Fruit and vegetable contain only about 1% of lignans, but they are also significant sources of lignans because they are consumed in higher amounts than seeds. 7-hydroxymatairesinol is the main representative of lignans. It is a white powder with great health benefits and it is present in the knots of coniferous trees, especially in knots of spruce. Lignans were extracted from the knots and used for fortifying fruit and vegetable spreads. Subsequently, the fortified products became subject to sensory analysis, their antioxidant capacity was measured by the FRAP method, total polyphenols content was found and lignan content determined using the HPLC method. The aim was to enrich commonly consumed foods with healthy lignans to avoid negative effects on the sensory quality of these products by the bitter taste of the lignan extract. Of the tested foods, plum jam and red pepper paste are the best options as they best block the bitter taste of lignans. There was a positive increase in antioxidant capacity in food products fortified by the lignan extract. For plum jam, strawberry jam, strawberry spread and red pepper paste, the more lignans were added to the products, the greater was the level of antioxidant capacity. The highest antioxidant capacity was reached in samples with the added amount of 340 mg of lignan per kg of product. As with the antioxidant capacity, total polyphenols content is dependent on the quantity of added lignans. Plum jam is the only exception, for which there was no statistically evident difference between the doses of 170 mg and 340 mg of lignans per kg. The values of lignans measured for samples with added 340 mg of lignans per kg range from 313 mg to 339 mg. For samples with addition of 170 mg of lignans per kg the measured values range from 129 to 164 mg per kg. Although lignans are beneficial for health, they are not acceptable to deteriorate the taste of the product. The samples containing the highest dose of lignans, i.e. 340 mg of lignans per kg, were rated as the least acceptable by consumers. Evaluated as the most suitable in this regard was plum jam with a dose of 170 mg of lignans per kg of product where lignans were not found to possess a sensory effect on the acceptability of the product.

Keywords: lignan; polyphenol; antioxidant capacity; soft fruit products

INTRODUCTION

Lignans are bioactive substances and members of the group of phenolic compounds referred to as phenylpropanoids. Found in the plant kingdom, in higher vascular plants, including conifers, they are involved in the protection of plants from infesting by microorganisms, or from exposure to insects and belong to the most common secondary metabolites of plants. They are dimers produced through oxidative dimerisation of two phenylpropanoid units linked by central carbons of their side propane chains. Linking additional bonds under the involvement of propane segments of the molecule in various oxidation states gives rise to all the possible structural types of lignans. Subsequent transformation produces norlignans, or conifoids and neolignans. There are currently more than a thousand of known species and more are constantly being discovered (Peterson et al., 2010). Lignans act against several types of cancer, such as breast, uterus, prostate, and colon cancer. Dinkova-Kostova et al. (1996) report that lignans are applied as anticancer agents or phytoestrogens, have antioxidant, antiviral, antibacterial and insecticidal effects and, finally, they protect against

cardiovascular diseases. Lignans can penetrate cell membranes, thus influencing the various cell processes. Peterson et al. (2010) considers some sources of lignans functional food with their protective function. This study investigates whether addition of lignan extract to chosen foods increased the value of total polyphenols and antioxidant activity without negative influence on consumer acceptability of the enhanced food. Lignans belong to polyphenols which are related to sensorial qualities such as colour, bitterness, astringency, etc. in foods and beverages.

Lignans are naturally occurring compounds that are found particularly in foods rich in fibre. As reported by Slanina (2000), there are more than 200 lignans, originating from more than 70 families of plants. They were found in all parts of plants; of these, typical are primarily the bark and wood of trees and the resin (MacRae, Towers 1984). They occur in diverse seeds, legumes, nuts, fruits and vegetables (Slanina, 2000).

Humans receive most of lignans from beverages, particularly from coffee and tea (up to 37% of total intake). In fruits, lignans are present in very low rates

ranging from about 1% of total solids (Johnsson et al., 2002). Despite this, however, fruits and vegetables are ranked among the significant sources as they are consumed in greater quantities than seeds (Landete, 2012; Haramatha, 2005). Hussain et al. (2006) were adding flour of linseed into biscuits to increase their quality parameters, e.g. the content of lignans and fibre.

Lignans also occur in heartwood of trees, particularly in the woody species that feature soft wood. For species of trees of hardwood type, they contain mostly flavonoids. Pine wood is rich in stilbenes. Holmbom et al. (2003) found that knots of trees contain 5% – 10% lignans. Levels of lignans reached in knots of the Norway spruce (*Picea abies*) ranged from 6% to 29%; of these, 7-hydroxymatairesinol (HMR) was the most represented member, which accounted for 85% of total lignans. Supplements based on this substance (i.e. HMR) and available on the market include HMRlignan™ and others. According to Taskinen et al., (2004) and Brusentsev and Eklund (2015) HMR has significant biological effects and is generally an anticancer and antioxidant agent. It also acts against hormone-dependent diseases. Willför et al. (2005) suggest that there are significant differences in terms of lignan content in knots obtained from a single tree.

Due to the beneficial and demonstrable health effects and the low content in foods the aim of this study was to extract lignans and add them to food. The research investigating the lignan levels in knots of conifers formed the basis for producing an alcoholic extract from such knots; the lignans generally contained in the substance include 7-hydroxymatairesinol (HMR) and α -conidendrin. The extract was added into selected types of intermediate products made of fruits and vegetables in order to increase the content of lignans and heighten the antioxidant capacity of the above.

MATERIAL AND METHODOLOGY

Chemicals for extraction: ethanol. for analysis: 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox); 2,4,6-tris(2-pyridyl)-s-triazine; hydrochloric acid; acetic acid; iron trichloride; sodium acetate trihydrate. Folin-Ciocalteu reagent; gallic acid monohydrate; sodium carbonate.

For HPLC analysis: methanol; acetonitrile; ortho-phosphoric acid; and formic acid.

Preparation of food products – the procedure: Knots of the Norway spruce (*Picea abies*) were milled using Cutting Mill SM 100 (Retsch, Haan, Germany). Lignans were extracted from the obtained chips by water and ethanol under the conditions as described in the design of the application for national patent No. 2014-870 (Híc et al., 2014).

The extract contained mainly 7-hydroxymatairesinol (HMR) and a low level of α -conidendrin (CONI) and was used to fortify plum jam, strawberry jam, and strawberry/blueberry spreads intended for adding to yoghurt and bakery products. The test also involved a red pepper paste, which is added to milk-based spreads. Three lignan doses were selected, i.e. 0; 170; and 340 mg and expressed as HMR per kg of the product. Five kinds of spreads and 3 lignan doses were used. Measurement of

antioxidant capacity, total polyphenols content and lignan content was done 3 times. After fortifying, the fruit and vegetable products were pasteurised at 85 °C for 20 minutes and stored for 1 month once modified as above. Subsequently, sensory analysis of samples was carried out and the antioxidant capacity, the total content of polyphenols and lignan levels determined.

Modification of food products prior to the chemical analysis: First of all, extracts were made from the samples to determine the antioxidant capacity, the total polyphenols content and the lignan level taking the steps according to Wicklund et al. (2005). Five grams of the sample were homogenised with a small amount of methanol (100%) using a grinding mortar. The process took place 1-2 minutes. The sample was then quantitatively transferred into a volumetric flask of 50 mL, which was filled with methanol up to the mark. The flask with the sample was placed into an ultrasound device for 15 minutes. The sample was transferred into a centrifuge tube and centrifuged 5 minutes at 3,000 rpm.

Antioxidant capacity: Antioxidant capacity was determined by the FRAP method, which is based on the reduction of ferric complex TPTZ using potassium ferricyanide or possibly ferric chloride; almost colourless substances, they produce colour complexes of iron and after the reduction that can be measured using a spectrophotometer. The determination made use of 23 mM sodium acetate in the solution of 34 mM acetic acid. The reaction mixture contained 12 mM FeCl₃, 10 mM 2,4,6-tri(2-pyridyl)-s-triazine in the solution of 40 mL HCl and a buffer, the ratio of 1 : 1 : 10. Two mL of the reaction mixture were mixed with 25 μ L of a sample diluted with distilled water using a plastic cuvette. The obtained solution was measured after ten minutes using Helios β spectrophotometer at a wavelength of 593 nm. Antioxidant capacity was calculated from the calibration curve for Trolox.

Total polyphenols: Total polyphenols (TP) content was determined by the method using the Folin-Ciocalteu reagent. Using a 50 mL volumetric flask, 0.5 mL of the sample was mixed with 20 mL of distilled water and 1 mL of the Folin-Ciocalteu reagent. The flask was shaken and after 3 minutes 5 ml of 20% Na₂CO₃ were added. After mixing, the flask was filled to the mark with distilled water. After 30 minutes the sample was measured at a wavelength of 700 nm using Helios β spectrophotometer. Total polyphenols content was calculated from a calibration curve for gallic acid.

Lignans: Concentrations of lignans, i.e. 7-hydroxymatairesinol (HMR) and α -conidendrin (CONI), were assessed by HPLC using an HP apparatus (Hewlett Packard 1050) with a diode array detector (DAD Agilent G1315B), and the Phenomenex Luna C18 column (2) (3 μ m, 2 x 150 mm). The mobile phase consisted of water - acetonitrile - o-phosphoric acid. Mobile phase A consisted of 5% acetonitrile +0.1% o-phosphoric acid; mobile phase B consisted of 80% acetonitrile +0.1% o-phosphoric acid. Used for separation was the gradient from 20% B to 80% B within 20 minutes, the flow rate was 0.25 mL/min.

Column temperature was 25 °C. HMR and CONI were detected at 220 nm.

Sensory analysis: Nine trained assessors took part in the sensory analysis within the scope of ISO 8586 requirements; the procedure was underway in the sensory laboratory of the Faculty of Horticulture Lednice that meets the ČSN EN ISO 8589 Standard. The method used for assessment was one according to the graphic scale (ISO 4121). The results were recorded on an unstructured graphic scale, the length of 100 mm. The assessors were asked to taste the samples and indicate their perceived intensity of a bitter and an astringent taste, and the consumer acceptability, where applicable, using the graphic scale. When evaluating, a distance was measured between the marks assigned to the sample by the assessor and the beginning of the scale, with 1 mm = 1 score, meaning that the person was able to give at least 0 scores and a maximum of 100 scores.

Statistical evaluation: The results were processed by the statistical program Statistica 10.0. Test of homogeneity was done followed by parametric analysis of variance (ANOVA). Tukey's LSD test with level of significance 0,05 was done from post hoc test (Table 1).

RESULTS AND DISCUSSION

The values of antioxidant capacity measured by FRAP were dependent on the content of lignans in samples of products (Figure 1). For plum jam, strawberry jam, strawberry spread and red pepper paste, the more lignans were added to the products, the greater was the level of antioxidant capacity. The highest antioxidant capacity was reached in samples with the added amount of 340 mg of lignan per kg of product. Conversely, the lowest antioxidant capacity was found in samples to which no lignans were added. All the differences are statistically significant ($p = 0.05$), except the blueberry spread.

Different results were probably due to the relatively high antioxidant capacity of the spread alone. Similar findings were reported by Balík et al. (2014), where the antioxidant values of grape juices were under a major impact by the manufacturing technology rather than by the addition of lignans.

As with the antioxidant capacity, total polyphenols content is dependent on the quantity of added lignans. Plum jam is the only exception, for which there was no statistically evident difference between the doses of 170 mg and 340 mg of lignans per kg (Figure 2). Since lignans are among polyphenols and feature antioxidant capacity, the conclusions presented are in accordance with assumptions. Samples that contained most of polyphenols were those fortified with 340 mg of lignans per kg of the product while the lowest values of total polyphenols were measured for samples to which lignans were not added at all. The graph shows that the addition of lignans increases the total polyphenols content in the samples. The lignan content in the products was tested by HPLC. It is apparent that the clean samples contain no lignans (Figure 3). The values measured for samples with added 340 mg of lignans per kg range from 313 mg to 339 mg. For samples with addition of 170 mg of lignans per kg the measured values range from 129 to 164 mg per kg.

The addition of the lignans extract was assumed to bring in food adverse secondary organoleptic characteristics such as bitterness or astringency. Although lignans are beneficial for health, they are not acceptable to deteriorate taste of the product. Due to the fact above, some products were assessed as unsuitable for fortifying using a lignan extract (Figure 4). When conducting sensory assessment of consumer acceptability, samples containing the highest dose, i.e. 340 mg of lignans per kg, were rated as the least acceptable by consumer. Evaluated as the most suitable in this regard was plum jam with a dose of 170 mg of lignans per kg of product where lignans were not found to possess a sensory effect on the acceptability of the product while

Table 1 Evaluation of antioxidant capacity, total polyphenols and sensory assesment of foods enriched with lignans.

Sample	Antioxidant capacity(FRAP) (mmol Trolox.kg ⁻¹)	Total polyphenols (mg.kg ⁻¹)	Sensory assesment (points)
Plum jam 340	8.48 g	1963.86 h	60.33 b c
Plum jam 170	7.41 f	1893.07 h	72.33 f
Plum jam 0	6.05 d	1661.23 g	72 f
Strawberry jam 340	8.21 g	1465.22 f	71.67 f e d
Strawberry jam 170	6.94 e	1313.38 ed	74.78 f
Strawberry jam 0	5.7 dc	1170.65 c	75.33 f
Strawbery spreads 340	7.01 e	1345.18 e	46.78 a
Strawbery spreads 170	5.33 c	1099.13 b	63 d c
Strawbery spreads 0	4.31 b	881.33 a	71 e f
Blueberry spreads 340	14.15 i	2628.88 k	49.44 a b
Blueberry spreads 170	12.74 h	2446.68 j	62.44 d c
Blueberry spreads 0	13 h	2308.88 i	72.95 f
Red pepper paste 340	5.98 d	1293.75 d	61.33 e d c
Red pepper paste 170	4.42 b	1108.84 b	61.22 e d c b
Red pepper paste 0	2.97 a	871.79 a	68 f e d c

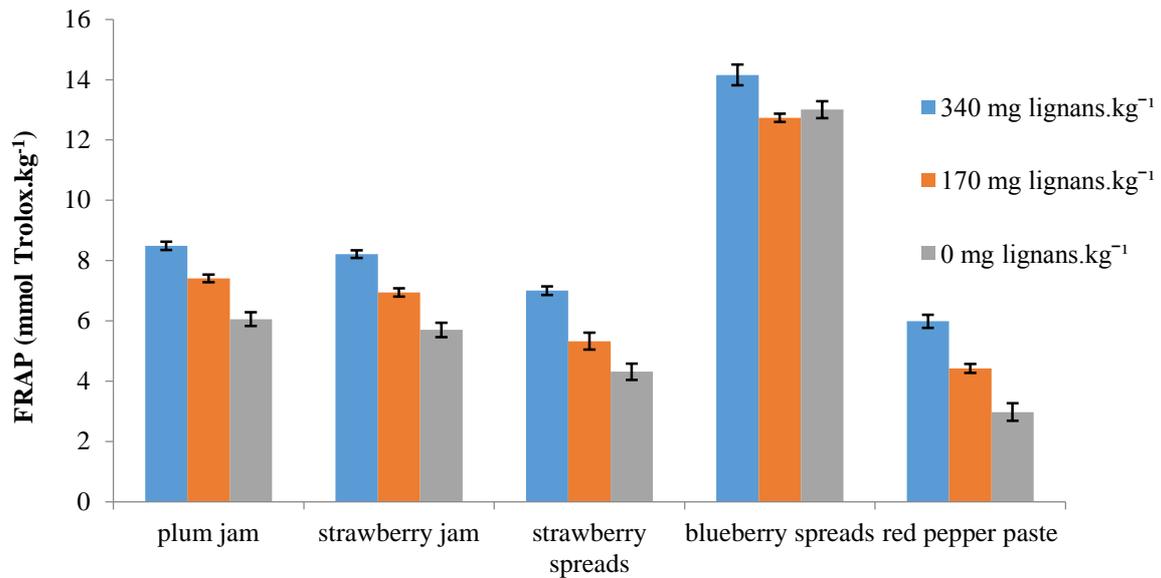


Figure 1 The values of antioxidant capacity for lignan-fortified fruit and vegetable spreads (FRAP method).

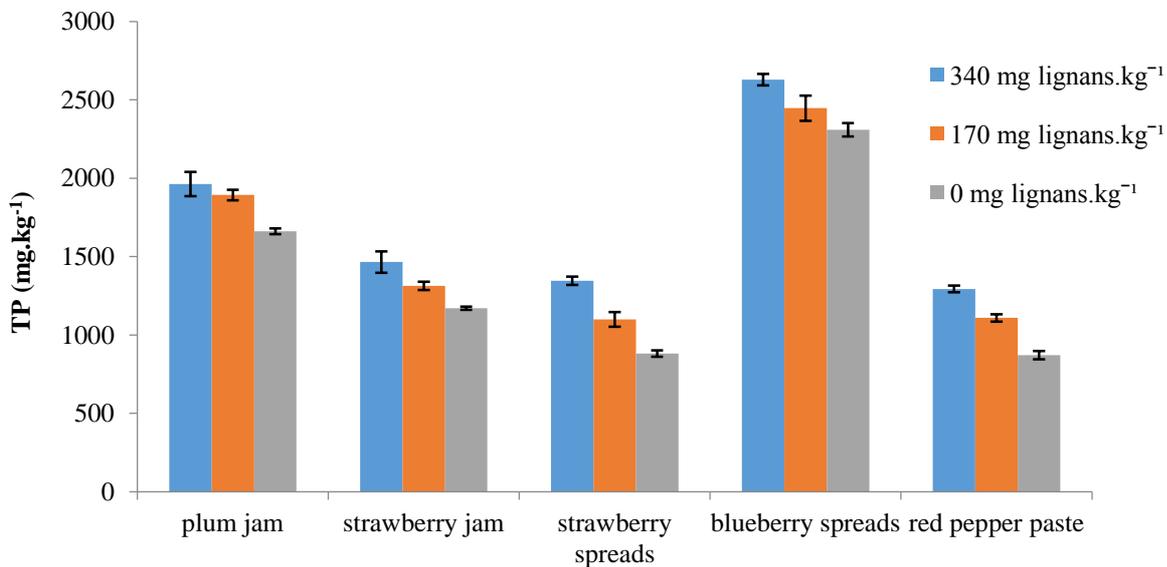


Figure 2 The values of total polyphenols for lignan-fortified fruit and vegetable spreads.

any higher dose caused a decline in consumer acceptability. A similar trend was recorded for strawberry jam, where no statistically significant difference was detected between samples ($p = 0.05$). For strawberry spread, a statistically significant difference was found between the sample containing 340 mg of lignans per kg and the sample containing 170 mg of lignans per kg and the one with no added lignans. When evaluating the red pepper paste, no statistically significant difference was found between the samples. Possible reason is that tasters might not be able to recognize difference among variants due to high pungency. The suitability also applies to the red pepper paste which, given its pungent taste, covered up the taste changes (Figure 4). Similar experiment was done

The study states that lignans HMR and CONI are the most present lignans in wooden chips of spruce tree. Several doses of wooden chips were added to white and red grape musts which were then pasteurised and evaluated after some time of storing. The results showed that

in the year 2008 by **Perlman et al. (2008)** who extracted grape pomace. They proposed the fortification of grape juice with the grape pomace polyphenol extract. The new beverage was tested at different extract concentrations for sensorial acceptance and antioxidant activity. An unacceptable astringency was noticed at 4% with antioxidant activity increasing three times. **Draijer et al. (2009)** added a mixture of red wine and grape polyphenols to a soy drink. Subsequently the enhanced beverage was given daily to 35 males with positive impact on the blood pressure. Similar research was done by **Novotná et al. (2016)** who increased the content of lignans 7-hydroxymatairesinol (HMR) and α -conidendrin (CONI) in grape musts by adding of spruce chips. enrichment by spruce chips increased the antioxidant capacity and also the amount of total polyphenols in the samples. The woody aroma and woody taste of the samples were increased simultaneously as sensory assessment showed. **Balík et al. (2016)** performed an

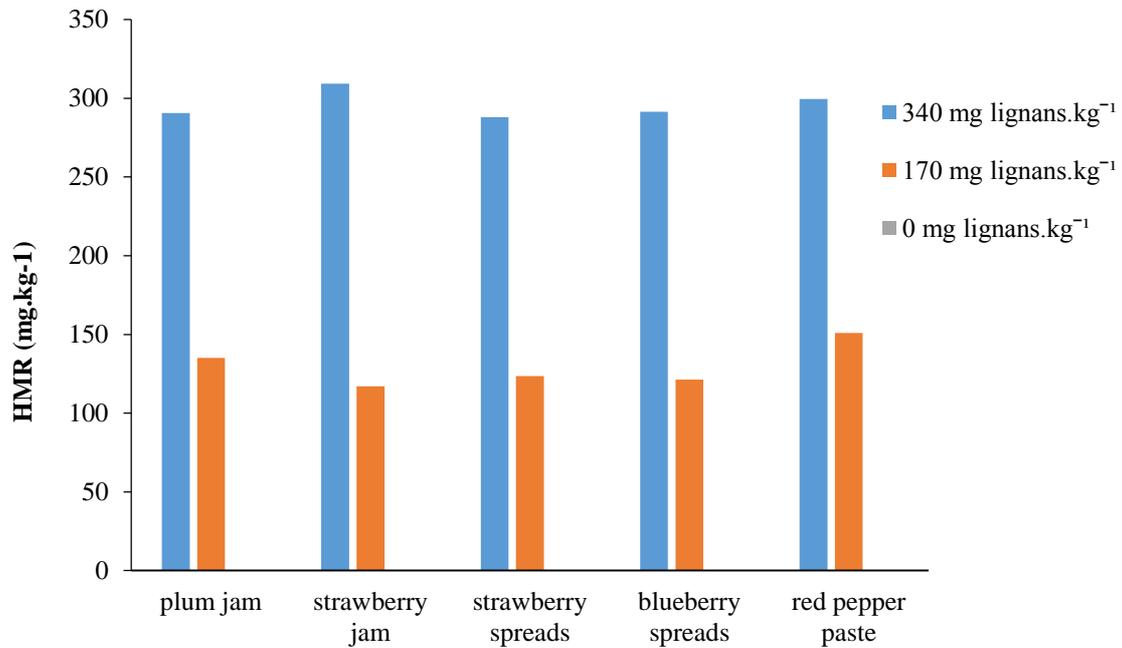


Figure 3 The lignan content in fruit and vegetable spreads.

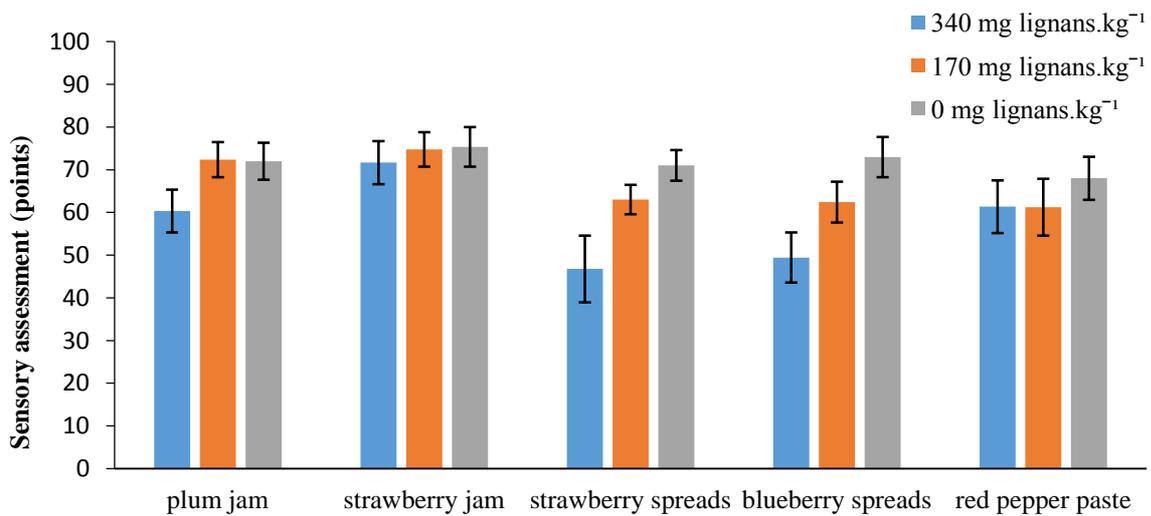


Figure 4 Sensory assessment of the consumer acceptability of food products depending on the addition of lignans.

experiment when lignan extract from wooden chips was added to white and red wines. Antioxidant capacity and total polyphenols content were measured after different times of storing. Increase of lignan content was observed in all samples after adding of the extract. The lignan content was stable even after 13 months of storing. Antioxidant capacity and total polyphenols content were not significantly influenced by extract enrichment. Kapoor and Ranote (2016) added the extract from jamun fruit of *Syzygium cumini* into pear juice in order to increase the antioxidant capacity. The products were sensorically evaluated and the results showed that juice with 4% of jamun phenolic powder reached the highest sensory evaluation. Enriched juiced showed about 9.24% higher content of total polyphenols than juices without powder enrichment. Antioxidant capacity was higher about 18.13%.

Storage period of 6 months caused significant decrease of bioactive compounds and antioxidant activity in supplemented pear juice. Liu et al., (2016) investigated the effect of oak chips on evolution of phenolic of bog bilberry syrup wine during bottle-aging. Results showed that the oak chips treatment significantly increased the content of phenolic compounds. The content of total polyphenols and antioxidant capacity decreased during 6 months of aging. Massini et al., (2016) increased the antioxidant capacity and total polyphenols of vegetable juices. They made an extract of apple peels which was rich especially in flavan-3-ols, flavonol glycosides and dihydrochalcones. Results showed that the addition of apple peel phenolic extract led to significantly higher radical scavenging capacity and to an increased protection against lipid peroxidation compared to control.

CONCLUSION

Lignans, as health-promoting substances, possess a great potential; their consumption is, however, low within the population since their levels are not high in common foodstuffs. For this reason, the exploitation of unconventional sources of lignans presents an attractive way of sourcing and further use. Extraction methods ensure that the substances can be sourced from potential waste materials such as wood chips. Application into foods, however, is often associated with changes in the sensory quality of the foodstuff as a result of the bitter flavour of the extract. It is therefore appropriate to add lignans into foods that are distinctive in terms of taste.

The results of evaluating the consumer acceptability show that the addition of 340 mg of lignans per kg of product is the least acceptable from a sensory aspect. In such samples, the assessment ranged from 46.7 to 71.6 scores out of a total of 100. In terms of consumer experience, the samples containing 170 mg of lignans per kg were almost as acceptable for the assessors as were the control samples with no addition of lignans. In samples containing 170 mg of lignans per kg the scoring ranged from 61.2 to 74.7 out of a total of 100. In terms of added lignans, strawberry spread intended for adding to yoghurt was the least suitable while in strawberry jam both of the lignan doses were rated positively. Addition of lignans into plum jam is the best when the amount is 170 mg per kg, as with the blueberry spread. For the red pepper paste, both lignan doses were rated almost equally and reached over 60 scores out of 100. In food products fortified by the lignan extract there was a positive increase in antioxidant capacity. Of the tested types of fruit and vegetable spreads, plum jam, strawberry jam and red pepper paste are the most suitable materials, their naturally distinctive flavour overlapping the taste of lignan extract being the very reason. mg.kg⁻¹

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