EFFECTS OF WATER EXTRACT FROM *Olea euopeae* ON THE SHELF LIFE OF MICROBIOLOGICAL AND SENSORY ATTRIBUTES OF WHEAT BREAD

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ABSTRACT

Mould growth is one of the major factors limiting the shelf life of bread. Olive leaves and their phenolics, which have antimicrobial and antifungal activity, have been shown to inhibit or delay the rate of a range of microorganisms, thus they might be useful as an alternative food additive.

In this study, the impact of olive leaf water extract (OLWE) at different concentrations (1, 5, 10, 20 and 40%) as a natural preservative have been assayed on the prevention of fungal spoilage of wheat flour bread during the storage periods. All packaged bread samples were stored at 20±2 °C and the microbiological analyses (total viable, total yeast-mould and lactic acid bacteria count) were performed.

The total yeast-mould (TYM) counts of control samples increased to 5.28 log cfu/g, while those of containing 10% or higher concentrations of OLWEs were lower than 3 log cfu/g (below 1.25 log cfu/g), end of the 6 days of storage. As a result, the OLWEs positively affected the microbiological shelf life of wheat breads stored at 20 °C, when compared to the control samples.

Key words: olive leaf extract, wheat flour bread, microbiological shelf life, sensory attribute

INTRODUCTION

Extended shelf life of bread may be obtained by the inclusion of a single ingredient or process change or a combination of many alternative changes according to food legislation, ingredient availability and its cost, consumer acceptance and social trends [1]. Increased shelf life of bread and other baked products can improve the productivity and profitability of a company allowing important expansion of bread lines with simultaneous economy in production, stocking and long distance distribution of higher quality products [2].

Deterioration of bread includes physico-chemical changes, moisture loss/gain and microbiological spoilage by such bacteria as *Bacillus subtilis* and *Bacillus pumilus* (rope) and some fungi species growth (*Rhizopus, Aspergillus, Cladosporium, Mucor, Penicillium and Neurospora)* are major factors limiting the shelf life of bread [3-5]. The most common source of microbial spoilage is mould growth [1, 2, 6-8]. Mould growth is frequently considered the foremost limiting factor and its prevention or delay leads to an extension of the shelf life of bread [2, 4, 9, 10]. These deteriorations can be delayed by: the addition of fungal inhibitors such as, propionic, benzoic, and sorbic, dimethyl fumarate, acetates, ethanol; by using a modified atmosphere packaging; by using pasteurization; by irradiating a product with infrared rays, microwaves or ultra-violet irradiation; and by freezing [2, 6, 8, 9-20]. However, the development of resistance in fungi against most drugs has been reported and this has created a need to identify new components that could be investigated in order to develop ideal anti-fungal formulations [21].

Commonly, a shelf-life around 2-4 days may be expected when bread is unpreserved [17, 20]. Therefore, various preservative agents are added to the bread formulation to prevent fungal spoilage [3, 5]. Apart from the repelling sight of visible growth, fungi are responsible for off-flavour formation. Moreover, the evidence that moulds produce mycotoxins and allergenic compounds generates risks to public health. These compounds may be formed even before the mould growth is visible [8, 10 ,11, 17, 18, 22-24]. During the bread making process, viable vegetative moulds and mould spores are usually destroyed by the heat of the baking process. However, mould spoilage may occur post-baking (even if a sanitation program is adopted) from the mould spores present in the atmosphere and during handling operations such as cooling, finishing and packaging. Airborne distribution of flour dust and mould spores is likely to give rise to contamination of the bread surface and is aggravated by high temperature and humidity [1, 2, 4-7, 10, 11]. The use of preservatives in bread is common and extensive because of their effectiveness in preventing or inhibiting microbial spoilage in general and mould growth specifically, although 'healthy, additive-free' products are more attractive [1].

The antimicrobial properties of plant molecules and their derivatives have been known and the commercial potential for their use has been discussed for a long time [25]. Plants, spices and herbs have emerged as effective compounds to ensure microbiological safety of foods and several studies have reported results on their preservative action [18, 26-29] and can be used as safe and effective alternatives to synthetic preservatives [27, 30-32]. Olives, olive oil, and olive leaf extracts are some of these foodstuffs with recognized medicinal benefits and food preservation properties dating back to the Egyptian empire [30]. Olive leaves are a copious by-products derived from olive tree cultivation, during pruning of the trees, harvesting and olive mills [33]. The olive tree (*Olea europaea* L., Oleaceae) is known for its capacity for making organic matter which contains a high proportion of biophenol compounds (BPs). These molecules accumulate essentially in the fruit (and leaf), especially during growth and the first stages of maturation [34, 35] and they are present in significant amounts [36, 37] which makes utilization of the leaves worthwhile [38]. Recently, high demand of whole olive leaf and olive leaf extract has increased for use in foodstuff, food additives and functional food materials and their

nutritive values [39]. Olive leaf extract, is a dark brown, bitter-tasting liquid derived from the leaves of the olive tree [40], has been used as folk medicine [15, 41-44]. The leaf extract contains high quantities of phenolic compounds [15,38,41-44] and olive leaves and their phenolic compounds have been shown to inhibit or delay growth of microorganisms [15-16, 26, 30, 38, 41, 45-48], thus they might be useful as an alternative food additive [45].

The objective of this study was to evaluate (I) the use of water soluble extracts of olive leaves for extending the shelf-life of wheat flour breads and (II) the effect of its on wheat flour bread sensory properties.

MATERIALS AND METHODS

Materials. For bread manufacturing, the wheat flour (*Triticum durum*) (Type 550) containing 12% protein (dry basis), 0.55% ash (dry basis), 14% moisture and 27.2% wet gluten was used and supplied from the Toru Flour Milling Co., Ltd (Bandırma/Turkey). Commercial compressed bakers' yeast (3.0%, w/w, flour basis) and salt (1.5%, w/w, flour basis) was used to prepare the bread dough.

Preparation of olive leaves water extract (OLWE). Olive leave samples of Cv. Gemlik were collected in June, 2010 from an olive grove located in Erdek, Northwest Turkey. The orchard has a planting density of 5x5 m., the trees are 20 years old and no phytosanitary treatments had been applied in the last year. The leaves were collected by hand, washed with deionized water and dried at 37 °C for 3 days. Then they were put in light protected glass bottles and kept in the refrigerator. Water-soluble extracts were prepared as described by Pereira *et al*. [46], with some modifications. Forty grams of dried olive leaves were homogenized with 50 mL of distilled water in a blender (Moulinex Masterchef 70, France). The ground material (40g) was added to 50 mL of boiling distilled water, boiled 45 minutes, then infused at room temperature for 10 min under stirring conditions. The extracts were then filtered through Whatman no.41 paper (125 mmØ, Cat No:1441 125, Whatman International Ltd. Maidstone, England) and the obtained water extract used immediately for making bread. The other concentrations (1, 5, 10 and 20%, w/v) were prepared in a similar way.

Bread making and packaging. The experimental bread dough was manufactured by adding 1, 5, 10, 20 and 40% of OLWE (60%), baker's yeast (3%), salt (1.5%). Bread dough without water extracts of olive leaf was the control. Dough was mixed for 10 min. in high-speed mixer (Diasno-Sp12d, Germany). Final dough temperature was in the range of 26±2 °C. The dough was rested in bulk for 15 min, scaled into 470 g portions, moulded, placed in pans and put into the proofer set to 32-34 °C and 85% relative humidity for 90 min. The pan size was 98 mm x 280 mm x 80 mm. The baking was carried out at 230 °C for 25 min. The oven (Werner-Pfleiderer, Carat 6.8, Germany) was pre-steamed before (0.3L water) and after loading (0.7L water) via the injection of water. The loaves were left to cool at room temperature (20±2 °C). After cooling, the loaves

were packaged by PE (Polyethylene) bags and closed with clips. All packaged bread samples were stored at 20±2 °C and 60±2% R.H.

Chemical analysis. In order to characterize the olive leaf, chemical analyses were according to Association of Official Analytical Chemists [49] procedures. Moisture content was measured by gravimetric method at 105 °C up to constant weight (24h) and was expressed in wet matter (g/100 fresh leaf). The total phenol content was determined according to the Folin-Ciocalteu method [50]. The results are expressed as mg gallic acid per gram of dry olive leaf. The phenolic compounds were analyzed using HPLC and oleuropein, verbascoside, hydroxytyrosol, and tyrosol were used as references compounds [51]. Individual phenols are expressed as mg per kg of dry olive leaf.

Microbiological analysis. Yeast and mould counts were examined with the method given by Rodrigez *et al*. [52] and Pascall *et al*. [53]. The microbial analyses were performed in triplicate after 0, 2, 4, 6 and 8 days of storage. The samples of wheat bread were weighed aseptically (10g) and homogenized in a Stomacher (Masticator, IUL Instruments, Spain) for 60 sec at room temperature (20±1 °C) with 90 mL sterile saline-peptone solution (8.5 g/L saline and 1 g/L peptone). Decimal dilution solution were prepared and duplicates of 1 or 0.1 mL of at least three appropriate dilutions were mixed or spread on the following agar media: Plate Count Agar (PCA; Oxoid CM0325) for total viable counts (TVC), incubated at 25 °C for 48 h; de Man-Rogosa-Sharp medium (MRS; Oxoid CM0361) for lactic acid bacteria (LAB), overlaid with the same medium and incubated at 30 °C for 48 h; Rose Bengal Chloramphenicol agar (RBC; Oxoid CM 549 supplemented with SR 78) for yeasts and moulds (YM), incubated at 25 °C for 72 h; The results of all microorganisms were expressed as log10 values of the colony forming units per gram (CFU/g) of wheat bread samples.

Table 1. Scores assigned for the evaluation of sensory attributes of the OLWE breads

Attributes	Scale anchors	
	Minimum (1)*	Maximum (9)
Bread volume	Not swell	Significantly greater than control
Crumb colour	Red	White
Crumb texture	Undeveloped	Homogenous
Crumb softness	Humid	Silky and soft
Crust colour	White	Bright brown
Crust texture	Hard	Smooth
Flavour	Bitter	Sweet-delicious
Mouth satisfaction	Pasty	Typical fresh bread
Overall acceptability	Dislike extremely	Like extremely

*1 is equal to "Dislike extremely", 5 is equal to "Neither like nor dislike" and 9 is equal to "Like extremely

Bread sensory evaluation. The sensory evaluation was carried out on the bread samples within 3-6h of baking. The bread samples were homogenously sliced and each assessor was provided with filtered water and asked to cleanse their palate between tasting. Sensory evaluation was conducted with 35 untrained assessors (18 females and 17 males), between 20-48 years of age. Panelists indicated their sensory evaluation for each attribute using a 9 point Hedonic scale (Table 1) ranging from 1 to 9. The sensory attributes were divided into nine groups corresponding to bread volume, crumb colour, crumb texture, crumb softness, crust colour, crust texture, flavour, mouth satisfaction, and overall acceptability. All samples were tested at room temperature under normal day light conditions. Sensory attributes including definitions were adapted form Meilgaard *et al* [54].

Descriptive microbiological and sensory evaluation statistical analysis. Results obtained for microbiological analysis and sensory attributes of OLWE breads were statistically analyzed with one-way Analysis of Variance (ANOVA). The mean values were submitted to the multiple comparison test using the LSD (Least Significance Difference) procedure that allows the attributes which differentiate the samples to be determined. The SPSS Professional Statistic package (SPSS 16.0 Inc, Chicago, IL, USA) was used for statistical processing of sensory data.

RESULTS AND DISCUSSION

Chemical composition of the olive leaf. Phenolic compounds of olive leaf are known to have diverse biological activities and may also be responsible for the pharmacological actions of olive leaf or, at least, for synergically reinforcing those actions [55]. In this study, the moisture content and total phenols of olive leaf was 47.55% (g/100g flesh leaf) and 12.285 (mg g^{-1} dry olive leaf), respectively. The oleuropein (11.850 mg g^{-1}) was found to be a major phenolic compound and the other phenolic compounds were hydroxytyrosl (406,00 mg g^{-1}), verbascoside (91,00 mg g^{-1}) and tyrosol (<1,8 mg g^{-1}). A lot of research studies evaluated phenolic contents and compositions of olive leaf extracted by several solvent methods and oleuropein was identified as a major phenolic compound [39, 46, 50, 56]. The results of this study is in good agreement with other studies by Briante *et al*. [57], Pereira *et al*. [46] and Malik and Bradford [58], in which oleuropein content of olive leaf extract from different cultivars, harvested at different times, was 60-90 mg/g, 1.05-14.35 mg/g, 26.47 mg/g, and 34.07-38.13 mg/g, respectively. Some authors pointed out that oleuropein content in *O. europaea* leaves is very low, around nd-173.35 mg/100g g dry material [39]. The contents of oleuropein in olive leaves was not changed by the collection period and/or in expanding periods of leaves [58, 59].

Microbiological results. Recently, interest in natural products or naturally derived compounds has been considerable in order to use extracts from these plants with antimicrobial activities to control pathogens or toxin-producing microorganisms in foods [26]. Olive leaves have been shown to inhibit or delay the rate of growth of a range of fungi; thus, they might be useful as a natural and an alternative food additive [26, 41, 48]. In this study, the impact of OLWEs at different concentrations as a natural

preservative has been assayed on the prevention of fungal spoilage of wheat bread. The most typical mycoflora causing bakery products' deterioration consists of xerophilic species of *Eurotium, Aspergillus* and *Penicillium* [27]. Fungal growth can be prevented by adding preservatives, but modern trend in the bakery industry is to reduce their application due to increasing interest of consumers for fresh and untreated products [60].

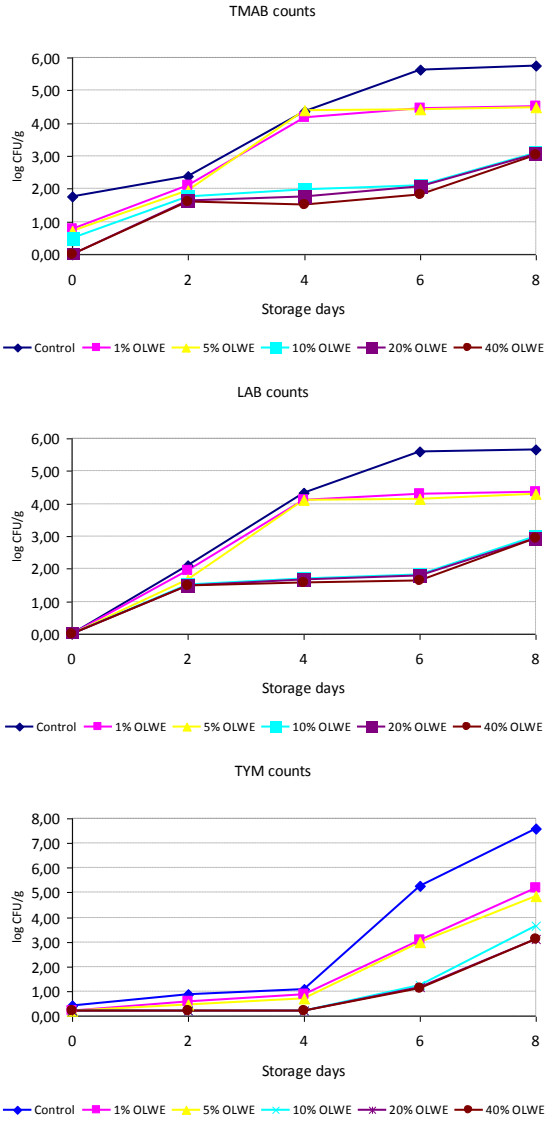

Figure 1. Microbiological changes of OLWEs added breads during the storage days

The total mould-yeast growth of wheat bread samples containing different OLWE concentrations stored at 20±2 °C is shown in Fig.1. The initial TYM counts of all the samples were within 0.2-0.4 log cfu/g, and were not significantly different at $p \leq 0.05$ level. During the 4th days of storage, the changes of TYM counts in all the samples were marginal, except control samples. Significant differences ($p \leq 0.05$) were found after 6 days storage. The TYM counts of the control sample increased to 5.28 log CFU/g, while those containing 10% or higher concentrations of OLWEs remained below 1.25 log cfu/g. Although the 5% OLWE showed lower counts than the control, the TYM count was not significantly different from that of the 1% OLWE containing sample ($p \leq 0.05$). On day 6, the TYM count of control sample increased 5 log cycles. In contrast, almost 1 log cycles increase in TYM counts were observed in the samples containing 10, 20 and 40% OLWE. Although the normal microbiological load for baked goods in routine quality control tests of the baking industry must be lower than 3 log CFU/g for moulds and yeast [61]. Control samples were kept for a prolonged time after exceeding the limit with the purpose of showing the extent of the OLWE effect. For TYM counts, OLWE increased the shelf-life of whead bread from 3 days to >6 days even at the 10% level. Filamentous fungi contamination reduces the time in which the bread can be consumed. Common rye or wheat bread can be stored for 3-4 days [18]. It was thought that for higher concentrations of OLWE, the shelf-life extension might be more profound.

In general, antimicrobial activities of spices are lower in foods than in culture media [62]. Korukluoglu *et al*. [26] determined that the aqueous extract of olive leaf showed the most prominent activity. According to those authors, aqueous olive leaf extract could be used by the food industry as antifungal agents as natural additives. However, fresh olive leaves are submitted under chemical and physical reactions and the high value compounds of post harvested leaves could thus be deteriorated. It is necessary to reduce the moisture content of fresh leaves to improve their shelf life, thus the drying process should allow not only the preservation of the leaves but will also improve or preserve their nutritive and functional values [63]. Brenes *et al*. [64] reported a significant decrease of o-dipenols after heating at 180 °C and related this reduction to thermal destruction or oxidative degradation of these compounds. Also Albi *et al*. [65] and Cerretani *et al*. [66] observed a greater decrease of total phenolic content in olive and virgin olive oil. Nevertheless, in this research, the olive leaves evaluated were heat-treated both during while obtaining extracts and also baking the wheat bread. Therefore, it is thought that the antimicrobial effects decrease because of heat treatment.

Sensory attributes. The radar plots of sensory attributes showed that the different levels of OLWE/wheat bread was generally accepted (Fig.2). The addition of the water soluble extracts of olive leaf seemed not to affect bread volume, crumb texture, crumb softness, crust colour, crust texture, mouth satisfaction and flavour attributes. No significant ($p<0.05$) differences were found between control and the other trials. The mean scores decreased with increase in the OLWE concentrations in colour attributes tested. As expected, the crumb colour of the 40% OLWE bread was significantly different (lower, 3.26±1.43) to the other samples. It was thought that the addition of increasing concentrations of olive leaf extract resulted the low colour score, due to dark colour of

their. Also, with respect to colour, the control and 1% OLWE sample did not show any significant difference from each other. The overall acceptability and flavour of bread was markedly more appreciated for of 40% OLWE (7.33±0,92 and 6.75±.,23, respectively)

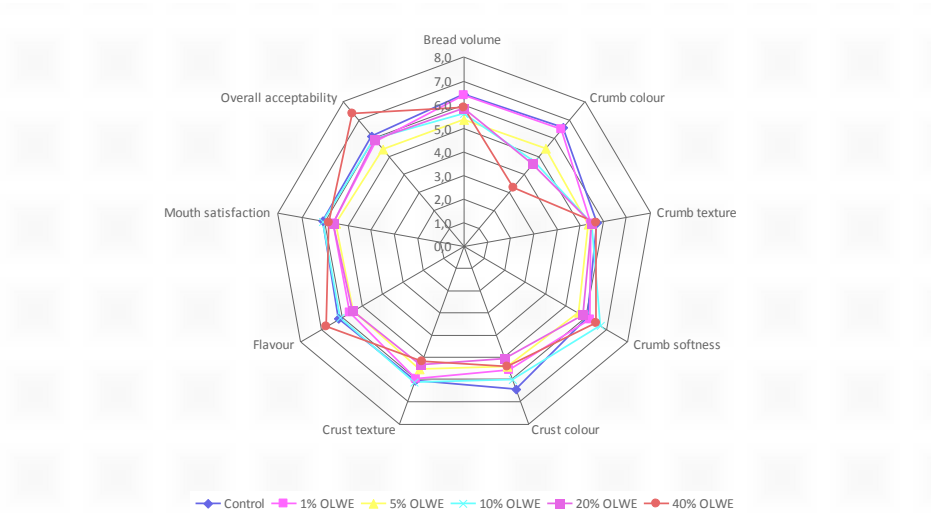

Figure 2. The radar plots of mean values for attributes differ among OLWEs added wheat bread

CONCLUSIONS

Using OLWE as an additive could increased the shelf life of bread (from 3 day to >6 day), nevertheless it was thought that the antimicrobial activity of OLWE decreased during the preparation of OLWEs and the thermal processing of bread production. Our results also demonstrated that with appropriate levels of addition, OLWE could lead to unfavourable change in the crumb colour of bread without causing significant changes in other sensory properties. Altogether, the 40% OLWE added bread showed the highest score for flavour and overall acceptability.

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