Journal homepage www.jzs.uos.edu.krd
Journal of Zankoi Sulaimani
Part-A- (Pure and Applied Sciences)

EFFECTS OF ROSEMARY (*Rosmorinus officinalis*) LEAVES AND ITS VOLATILE OILS ON WHEAT DOUGH PROPERTIES AND BREAD SHELF LIFE

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Article info

Original: 21 Feb. 2015
Revised: 16 Apr. 2015
Accepted: 19 Apr. 2015
Published online:
20 Sep. 2015

Key Words:
rosemary leaves, rosemary volatile oil, wheat dough rheology, dough fermentation, loaf shelf life

Abstract

The effects of ground rosemary leaves (2.5, 5, and 10%) and rosemary volatile oil (0.1, 0.2 and 0.4%), on wheat dough rheology, fermentation and its bread quality were studied. Amylograph results showed a decrease in pasting temperature from 63°C to 61.5°C in all VO doses. Also amylograph results showed ground leaves had an inhibitory effect on dough α-amylase especially at 2.5%. Farinograph results showed only 0.1% VO had a significant effect on farino parameters while other treatments generally had a softening effect. Dough fermentation values were decreased at all doses of ground leaves and VOs except for 0.2% VO which had an activation effect. Also the dough and loaf volume/weight were also decreased at all doses. The loaf shelf life was dose dependent and significantly increased against mould growth up to thirty days storage.

Introduction

Rosemary (Rosmarinus officinalis L.), is a member of Lamiaceae family, which grows worldwide and has been cultivated since ancient Egypt, Mesopotamia, China and India **[1]**. Rosemary is one of the common herbs used for seasoning and its extract has a wide application as a good source of natural antioxidant for its phenolic contents.

Rosemary leaves contain a number of potentially biologically active compounds, including rosmarinic acid and carnosic acid, in addition to other phytochemicals such as camphor, caffeic acid, ursolic acid, betulinic acid, rosmaridiphenol and rosmanol **[2]**and Basaga **[3]**.

Ground rosemary leaves are used in its own or with a mixture of spices, as a seasoning in some bakery products such as Kulecha (pie and cookies), a famous bakery product in Middle East including Iraq and Kurdistan. Effects of rosemary powder and its volatile oil in wheat flour may result in improving or dissatisfaction on the dough fermentation and rheological properties **[4]**.

Ozcan (2009) **[5]** reported that rosemary had improving effects on the extensograph and farinograph characteristics of wheat flour dough. Further, the effect of rosemary on wheat dough rheology and

fermenting ability were studied by **[6]** reporting on the loss of the CO_2 gas (mL) in dough samples with added rosemary (2.0% alcoholic extraction) using rheofermentometer analyses.
The purpose of this study was to evaluate the effects of ground rosemary leaves and its volatile oil on rheological and fermentation characteristics of wheat dough and the bread shelf life.

Material and Methods

Materials:

Wheat white flour (78% extraction) trademarked Jenan imported from National Milling Co. UAA, active dry yeast (Hasmaya), corn oil (Zer), full cream milk powder (Nido) and uniodised salt (Zer) were purchased from local super market Zara – Sulaimany – Kurdistan - Iraq. Clevenger, Brabender Amylograph (C. W. Brabender, Duisburg, Germany) and Brabender Pharinograph (C. W. Brabender, Duisburg, Germany).
The fresh green leaves of rosemary were obtained from the farmyard of the Faculty of agricultural Sciences - University of Sulaimani – Kurdistan - Iraq. All chemical reagents used in this study were Analar grade for scientific research.

Methods

Proximate analysis

Wheat flour was analyzed for moisture, protein, fat and ash according to the method Nos. 44-15A, 46-10, 30-10, and 08-01 respectively as described in AACC, (2000) **[7]**. While dried rosemary leaves was analyzed for moisture, protein, fat and ash methods Nos. 925.09, 920.87, 945.38F and 923.03 respectively according to AOAC (2004) **[8]**.

Extraction Volatile oils: Volatile oils were extracted by hydro distillation using Clevenger apparatus as prescribed in BSI method **[9]**.

Preparation of Rosemary leaf powder (RLP)

Rosemary leaves were washed and dried under current of air at room temperature about 22° C then grinded by coffee grinder, the powder were sieved by 150 μm sieve and the particles pass through the sieve were collected and used in the experiments.

Rheological characteristics

Brabender Viscoamylograph was used to determine the flour paste characteristics according to the method No. 22-10 as described in AACC (2000) **[7]**, additionally viscoamylograph was used to determine the peak viscosity at 50^{o} C and gel set back.
Rheological characteristics of flour samples were determined with Brabender Farinograph according to the method No. 54-21 as described in AACC, 2000 **[7]**. Dough characteristics such as water absorption, dough stability, dough development time, tolerance index and softening of dough were interpreted from farinogram.

Gassing power

Gas power as an indictor on dough fermentation was measured according to (AACC method No. 22-14.0.1) **[7]** measurement of gassing power by pressuremeter method (AACC, 2000) **[7]**. Ten g of flour samples with (7) ml distilled water , (0.2) g yeast and the studied concentration of RLP or RVO were added to form uniformly dough. Every (30) min of dough fermentation, the gas power was recorded until 3 hours.

Dough Volume

As the above method, the same amount of dough was put in cylinder instead of pressuremeter and the rising of dough will recorded.

Baking test

AACC (2000) method No.10-9 **[7]** was used to prepare pan bread (loaf). Full cream milk powder 4.0%, sucrose 4.0%, salt 1.5%, instant dry yeast 1.5%, corn oil 1% and water 65%, each of Rosemary Leaves Powder (RLP) 2.5, 5 or 10% and Rosemary Volatile Oils (RVO) 0.1, 0.2 or 0.4%based , on the flour weight, were added to the formula. Loaves weight and volume were determined after 20 min. of baking time and cooling using electric balance and rapeseed replacement respectively.

Sensory evaluation

Eight panelist evaluated the sensory characteristics applied to evaluate pan bread were, crumb color, taste, flavor and crust color. For excellent parameter 5 degrees was given, while for very bad character 1 degree.

Bread spoilage

All treatments of pan loaves stored separately in closed polyethylene bags at 22 ±3°C. The loaves were observed for mold growth on loaf surfaces on daily basis.

Statistical Analysis

All the data are means of triplicates with standard deviation (SD) and Least Significant Differences (LSD) for all treatments were calculated using SAS linear models procedure **[10]**.

Results and discussion

Chemical analysis

Proximate analysis of wheat flour showed the moisture, protein, hexane extraction, nitrogen free extraction and ash percentages were 12.5, 10.50, 1.00, 75.00 and 1.17% respectively (table 1). Bread may be made from all-purpose wheat flour, especially bread flour, containing protein (12–14%), is recommended for high-quality bread (such as pan loaf). However, low protein content flour (9 – 11 %) can also be used to produce bread **[11]**.

The percentage of flour ash (1.17%) was higher than the applied specification for all-purpose flour, which is recommended not to exceed than 0.5- 0.6% **[12].** The increasing of flour ash content may be due to the supplementation of used flour with iron salts, which haven't a noticeable effect on flour or bread color. Banu, et.al, (2012) **[13]** found a good quality of bread was produced when bran added to white flour to a level similar in whole wheat flour.

Hexane extract, protein, and ash percentages of rosemary leaves powder (RLP) were 10.7, 4.6, and 7.8 respectively (table, 1). The high percentage of hexane extract of RLP may play as an improving agent for dough and final products of breads. On the other hand, the addition of RLP may weaken the flour strength due to RLP's low protein and high ash content, resulting in gluten dilution. However, probably the presence of anti oxidizing agents in the RLP have led to minimize the dilution effect. The high ash content in RLP though is useful for improving bread quality, in the meantime high amount gives rise to an unacceptable appearance of the loaf crumb. Salts at low concentrations (less than 0.3M), has strong salting – out tendencies on gluten protein **[14]**, however salting – out by mineral salts causes strengthening and stability of gluten.

Table 1: Proximate analysis of wheat flour & Rosemary leaves powder

Samples	Moisture %	Protein %	Hexane Extraction %	Ash %	Nitrogen free extraction %*
Wheat flour	12.48± 0.38	10.5± 0.12	1.00± 0.03	1.17±0.09	75.00
Rosemary Leaves Powder	6.10± 0.25	4.63± 0.08	10.67± 0.09	7.81± 0.11	70.79

*Calculated by differences

Effect of RLP and RVO on dough rheology

Viscoamylograph parameters

Effects of RLP and RVO on flour paste properties determined by viscoamylograph (table 2 and figure 1) showed that, addition of each of RLP and RVO had a different effect on amylographical flour paste parameters. Pasting temperature (PT) for wheat starch showed no affect by any of the percentages of RLP, whereas the addition of all RVO doses had significantly decreased PT from 63°C to 61.5°C. RVO may have assisted the wheat starch granules to swell up faster than control while this effect was not observed in RLP

addition, probably due to their effect on decreasing wheat starch concentration, which alters starch PT. The consequent of the two opposite effects may explain why RLP doesn't effect on wheat starch PT.

It was observed the addition of 2.5 % of RLP had the highest effect on most viscoamylograph parameters comparing to other treatments (table 2). The effect of 2.5% dose of RLP was distinctively showing an increase in viscoamylograph parameter values (maximum resistance at heating, cooling and gel set back) that is possibly an indicative of RLP having an inhibitory effect on wheat flour amylases. This is line with **[15]** reported that the rosmarinic acid extracted from rosemary leaves had an inhibitory effect on pancreatic amylase. However, this effect was found to have an inverse relationship, i.e. the effect decreased with an increase in RLP doses. This phenomenon can be attributed to high ash and mineral content in RLP resulting in the consequent decrease of the total starch in the dough matrix, not due to decreasing of amylases inhibitory activity.

In contrary, the addition of RVO decreased viscoamylographical parameters values (table 2 and figure 1), probably due to the RVO role in acceleration of wheat starch to swell faster, resulting in breaking down the granules earlier than the controlled starch granules. Peak viscosity (PV) for wheat starch was 840, dropped to 780, 780, and 720 AU for 0.1, 0.2 and 0.4% of RVO addition respectively. Decreasing of PV values when VO was added theoretically indicates that α amylase was activated by the VO addition due to an inverse relationship between α amylase activity and amylograph PV **[16].** The higher values of cold paste and setback viscosity after VO addition are indicative of having no activation effect on α - amylase, therefore, the effect of VO on decreasing PV values is mainly due to physical effect as we mentioned earlier. However Davidović et al., (2010) **[6]** reported the addition of 0.5, 1, and 2% rosemary alcohol extract increased amylograph maximum viscosity attributing to the extract effect on decreasing amylolytic activity.

Table 2: Effect of Rosemary leaves powders or their volatile oils on viscoamylograph parameters

Sample	Pasting Temperature (^{o}C)	Peak Viscosity (AU)	Peak Viscosity (°C)	Hot Paste Viscosity (AU)	Break down viscosity (AU)	Cold paste viscosity (AU)	Setback viscosity (AU)
Control	63±0.00	840±1.34	95±0.58	2.80±660	180±1.77	1150±6.55	310±3.23
2.5%RLP	63±0.25	890±2.73	92±0.72	3.71±610	280±3.01	1360±4.19	470±3.24
5% RLP	63±0.15	850±3.00	91±0.44	±1.10680	170±2.04	1270±7.98	420±5.45
10% RLP	63±0.10	720±0.53	91±0.73	±2.33560	160±1.88	1060±3.24	340±2.33
0.1% RVO	61.5±0.00	780±2.73	94±0.01	±1.87640	140±2.61	1210±2.33	430±2.21
0.2% RVO	61.5±0.10	780±1.01	94±0.51	±2.98640	140±2.15	1250±6.11	470±3.5
0.4% RVO	61.5±0.00	720±2.30	94±0.34	±0.01580	140±1.71	1150±4.31	370±3.56

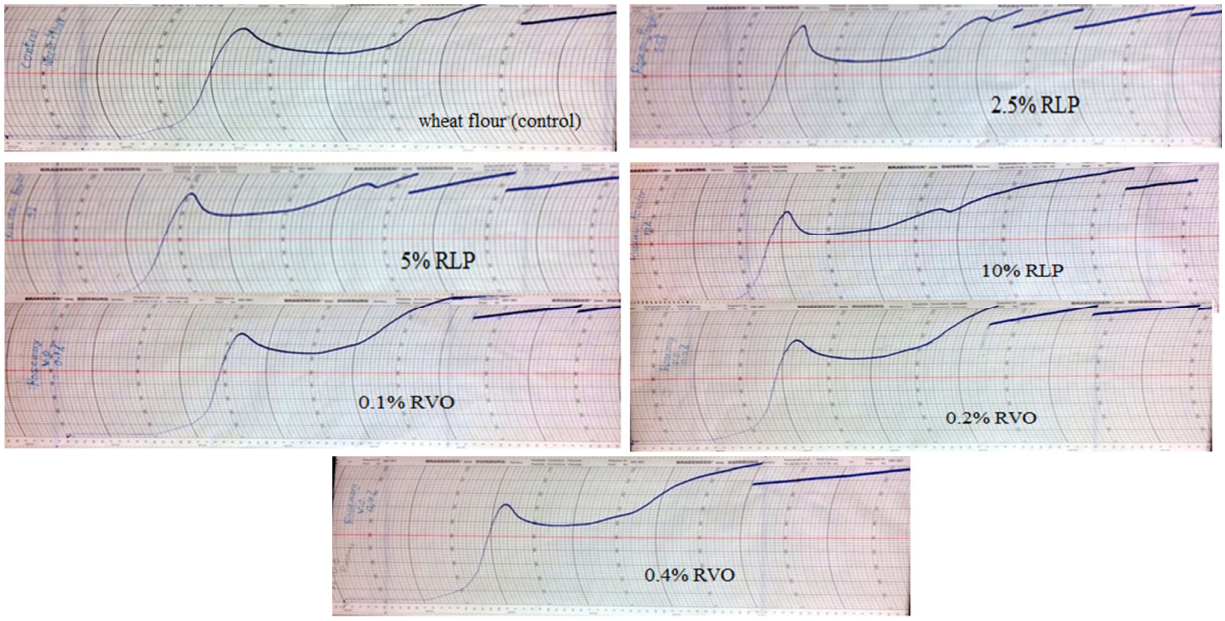

Figure 1. Effect of RLP and RVO addition on wheat flour viscogram

Farinograph

The RLP and RVO addition on wheat dough farinograph parameters showed different effects (table 3). RLP, besides to its VO content, have other chemical components that each has a different effect on dough farinograph parameters, while RVOs have the characteristics of the volatile oil compounds. The Farinograph showed an increase of flour water absorption is dose dependent with the increase of RLP. This effect might be due to the rosemary fiber content (contains about 19.40%) **[17]**, thus the volatile oil did not increase the water absorption. This is in line with **[18]** who reported that water absorption increase with increasing of fiber content. The effects of RLP addition showed an inverse relationship with all the Farinograph parameters except water absorption (table 3). This phenomenon may be attributed to the effect of nonvolatile rosemary components on disulfide bond formation. Whereas an increase with RVO addition caused a decrease of farinograph dough stability, indicating that RVO has a softening effect on the dough due to their antioxidant contents **[19].** It was reported by (Lillard, 1982) **[20]**, the presence of antioxidants might lower the ability of oxidizing enzyme system in dough to oxidize gluten sulfhydryl groups to disulfide bonds. However, addition of RVO up to 0.4 % did not show an effect on most farinograph parameters especially breaking down time values, which gives an indication on dough stability and their resistance to rupture.

Davidović et al., (2010) **[6]** found that, addition of 0.5% rosemary alcohol extract slightly increased farinograph dough stability while addition 1 and 2% of rosemary alcohol extract slightly decreased farinograph dough stability and degree of softening, however, these extract additions did not change the quality number of the dough on the Farinograph.

Table 3: Effect of Rosemary leaves powders or their volatile oils on farinograph dough properties

Samples	% Water absorption	Development Time (min.)	Stability (min.)	Tolerance index (FU)	Breakdown Time (min.)	Quality number
Control	66.1±0.78	10.7±0.05	12.7±0.81	43±1.20	14.0±0.31	140±3.1
2.5 % RLP	69.1±0.01	7.7±0.09	4.1± 0.44	111± 3.30	9.2±0.29	92±2.9
5% RLP	71.9±0.23	10.0±0.15	7.0±0.16	83± 3.12	12±0.09	120± 0.9
10% RLP	76.1±0.19	12±0.43	12.3±0.92	29±0.50	16.2±0.11	162±1.1
0.1% V.O	65.6±0.45	11.0±0.29	14.0±0.66	35± 0.25	15.0±0.07	150±0.7
0.2% V.O	65.2±0.31	10.2±0.71	11.7±0.28	36± 0.31	14.6±0.44	146±4.4
0.4% V.O	65.0±0.11	10.8±0.08	8.7±0.09	37± 0.19	15.0±0.14	150±1.40
$LSD_{(P\geq 0.05)}$	0.82	1.01	1.65	0.89	0.43	4.30

Effect of RLP and RVO on dough fermentation power

The effects of RLP and RVO addition on dough fermentation (table 4) showed an inverse relationship between the RLP and RVO doses and the dough fermentation power. However, RLP addition somewhat decreased the dough fermentation more than RVO did. RLP contains several phytochemicals such as phenolic compounds that could inhibit enzymes activities, which have an important role in dough fermentation such as amylases **[21]**. The results (table 4) showed that volatile oils, have a considerable effect on dough fermentation especially at % 0.4 which decreased fermentation power. Addition of RVO not completely inhibited yeast activity since it continued ferment the dough as it will be clear again from the results of dough volume (table 5) and baking test (table 6). It seem that, the effect of RLP and RVO on dough fermentation may be attribute to the volatile oils not to other phytochemicals because the effect of the higher concentration 10% of added RLP was similar to higher concentration 0.4% of added RVO. Also we can say that, the effect of RLP and RVO on dough fermentation may be attributing to their effect on the assistant material such as amylase more than on yeast, which like this effect can negligible.

Table 4: Effect of Rosemary leaves powders or their volatile oils on gas pressure mm/Hg

Time	Control	2.5%RLP	5%RLP	10% RLP	0.1% V.O.	0.2% V.O.	0.4% V.O.	LSD $_{(P\geq 0.05)}$
30	16.5±1.5	7.5±1.8	2.5±0.89	2.5±0.05	9±1.31	15±2.3	2±0.51	1.05
60	25.5±2.55	15±2.31	±3.0115	10±2.13	24±3.71	31±3.22	12±1.80	2.17
90	46±5.23	31±3.32	33±4.78	±1.819	37±4.81	49±7.80	20±2.42	2.88
120	59±4.55	49±1.89	47.5±2.87	20±3.11	55±1.15	68±4.84	23±2.99	4.63
150	77±5.44	64±1.55	63±4.79	25±1.01	66±5.11	80±4.85	28±2.25	5.98
180	91.5±7.03	73.5±2.23	78±3.17	30±0.56	80±6.71	95±5.97	35±1.89	7.16
210	104±6.99	92.5±4.31	92±5.99	35±1.96	88±5.94	111±7.73	41±3.02	6.56
240	118±8.73	108±4.99	105±6.01	40±3.22	97±6.04	127±8.12	47±3.11	7.97
$LSD_{(P\geq 0.05)}$	4.08	3.02	4.76	1.88	3.88	5.23	2.87	

Effect of RLP and RVO on fermented dough volume

Dough volume property depends mainly on two factors; fermentation power and dough capability to extant obeying to gas pressure. The fermentation power is discussed above, while the second factor is shown in table (2). The result shown is an inverse relation between the RLP dose increase with a decrease in dough volume, i.e. an addition of RLP resulted in a decrease of the dough volume sharply (30-42cm^3) comparing to control (60cm^3). This behavior may attribute to the presence of solid matter in RLP that could dissociate the gluten network causing loss of their capability to keep on gas and to extant to increase the dough volume. On the other hand, since the RVO is free from solid matter, the behavior of RVO addition on the dough volume was different and this is explicit in the results. Dough volume decreased by 10 cm^3 when RVO was added while RLP addition decreased the dough volume between 18- 30 cm^3 comparing to control. Conclusively, the addition of RLP showed an effect on dough mechanical properties (resistance to extension, Plasticity and elasticity) while RVO had insignificant effect on these properties.

Table 5: Effect of Rosemary leaves powder and its volatile oils on dough volume

Time: min.	Control	RLP 2.5%	RLP 5%	RLP 10%	RVO 0.1%	RVO 0.2%	RVO 0.4%	LSD $_{(P\geq 0.05)}$
0.00	15±0.00	15±0.00	15±0.00	15±0.00	15±0.00	15±0.00	15±0.00	-
180	60±2.61	35±2.21	42±3.5	30±1.88	50±2.89	50±3.10	50±3.77	2.17
$LSD_{(P\geq 0.05)}$	2.09	1.33	1.51	1.88	2.79	3.73	1.88	--

Effect of RLP and RVO addition on baking test

The effect of RLP and RVO addition on loaf characteristic (table 6) shown with an increase of RLP and RVO has resulted in a decrease of loaf characteristics (loaf volume and loaf volume to loaf weight ratio). The effect of RLP on loaf quality was more than RVO addition, probably due to the presence of chemical components in RLP that might softened the wheat gluten resulting in lowering their ability to extent. Although RVO has only volatile oils and its effect was less than RLP, however, the antioxidant effect of RVO may have delay gluten development and consequently decreased the loaf volume. Generally, results of this test may be agreed with dough fermentation and volume of dough tests, further using RVO is more favorable than RLP.

Table 6: Effect of RLP and RVO addition on loaf characteristics

Treatment	Dough weight (g)	Loaf weight (g)	Loaf volume (cm^3)	Specific volume
Control	150	140± 0.5	423± 11.06	3.02± 0.09
2.5% RLP	150	142± 0.8	332± 8.55	2.34± 0.12
5% RLP	150	142± 1.8	314± 1.99	2.21± 0.07
10% RLP	150	141± 2.01	v326± 6.34	1.91±0.34
0.1% RVO	150	141± 0.88	395± 5.17	2.80± 0.20
0.2% RVO	150	137± 1.85	381± 3.89	2.78± 0.38
0.4% RVO	150	140± 3.17	378± 7.18	2.70±0.28
$LSD_{(P\geq 0.05)}$	-	1.98	4.14	0.41

Effect of RLP and RVO addition on loaf sensory evaluation

Results (table7) showed the addition of RLP and RVO takes the same trend in decreasing loaf quality. Increasing of RLP addition had unacceptable effect on all sensory properties of loaf. Addition of 5% and

10% of RLP resulted in an entire refusal of the bread due to high content of phytochemical substances, however, the addition of 2.5% or less has offered a bread more acceptable. The addition of RVO showed less effect on the bread quality, in deed didn't effect on the crumb and crust color, but it had unacceptable effect on the bread taste and flavor. Addition of % 0.1 RVO may be the best dose with having no negative effect on crumb, crust, color and bread flavor. The slightly effect of RVO on bread taste was still acceptable (3.9 out of 5) comparing to control (4.3 out of 5).

Table 7: Effect of RLP and RVO addition on loaf sensory evaluation

Loaf Characteristics Treatments	Crumb color (1 -5)	Taste (1-5)	Flavor (1 -5)	Crust Color (1 -5)	Total 20 degree
Control	4.62± 0.03	4.37± 0.10	4.31± 0.01	4.37± 0.23	17.67± 0.12
2.5% RLP	3.43± 0.21	3.00±0.26	3.81±0.17	3.41±0.45	13.71±0.61
5% RLP	2.62± 0.19	1.46± 0.37	2.90± 0.11	2.28± 0.17	9.26± 0.36
10% RLP	1.37± 0.08	0.25± 0.17	3.50±0.21	1.50± 0.08	6.62± 0.24
0.1% RVO	4.25± 0.28	3.87±0.23	4.25± 0.31	4.37± 0.18	16.74±0.45
0.2% RVO	4.12±0.18	2.87±0.18	3.75± 0.12	4.12± 0.23	14.86±0.23
0.4% RVO	4.37±0.12	1.87± 0.09	3.25± 0.10	4.00± 0.31	13.89±0.65
$LSD_{(P\geq 0.05)}$	-	-	-	-	1.72

Control of loaf spoilage fungi by RLP and RVO

During one weak (7 days) the fungi growth started to appear on loaf surface (control) while didn't appear on any surface of the loaves treated with RLP and RVO for more than thirty days (figure 2). Suhr and Nielsen(2003) [22] reported that rosemary volatile oil had limited effects on rye bread spoilage fungi compared to thyme, clove and cinnamon oil as the best growth inhibitor. Further, **[23]**, correlated between the pH of media and volatile oils effect on fungi growth, stating that rosemary, thyme and bay were more effective at pH 5, but losing their activity as pH increased.

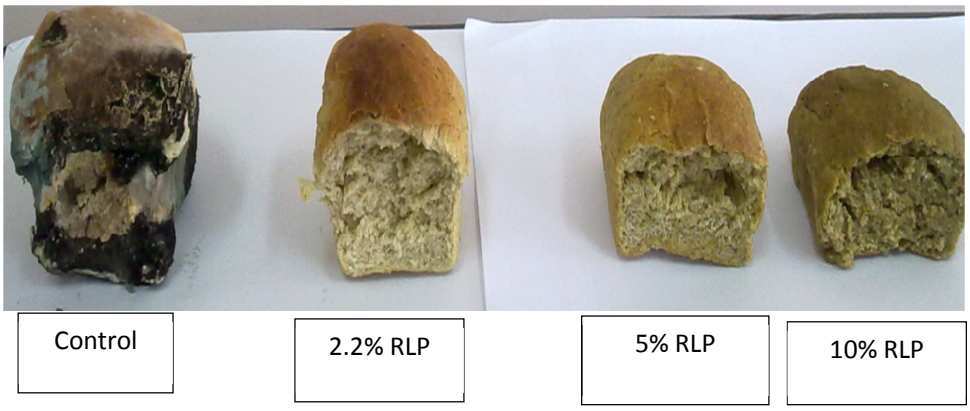

Figure 2: Effect of RLP addition on pan loaf shelf life after thirty days of storage

Conclusions

1. The wheat flour in this study was treated with RLP and RVO and the resulted bread quality can be explained by the interaction between chemical components of rosemary leaves and its volatile oil compounds with active materials of wheat flour. Oxidation of sulfhydryl to form disulfide bonds, activation or inhibition of flour enzymes, especially oxidative and hydrolytic enzymes, keeping the quality of bread to decrease staling and prevent fungi spoilage, are the most important factors that to be considered when a natural or artificial materials is added.
2. Rosemary leaves may contain amylase inhibitors in addition to some types of gums such as arabinoxylan that probably have caused to increase amylographic values at the low concentration, 2.5 and 5%. Whereas at higher concentration the amylographic values were clearly decreased as a result of starch dilution factor. On the other hand, volatile oil behaved as an activation agent to amylase, however, this effect disappeared when RLP was added probably due to the interaction among the opposite functions of their components.
3. Rosemary also contains anti-oxidants such as, carnosic acid **[24]** that could well prevent the oxidation of sulfhydryl groups leading to soften the dough. It was observed that Rosemary has caused softening of dough consistency as the results of the presence of an active antioxidant materials in rosemary leaves and oils. Ozcan (2009) **[**5] found that rosemary volatile oils had an advantage on wheat dough rheology but its effect was in the direction of dough softening when determined by extensograph. This finding emphasized that presence of anti-oxidant in RVO will delay dough formation and behaves as a reducing agent.
4. The addition of low concentration of RLP and RVO 2.5% and 0.1% respectively didn't show a significant effect on dough rheology and fermentation, further, these doses had a good ability to inhibit fungi growth without significantly affecting on baker's yeast growth.

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