

*Full Length Research Paper*

# Evaluation of the effect of precipitated whey protein (PWP) and fermentation time on the quality of Iranian Lavash bread

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Flat bread is the main staple food for most Iranians and Lavash is commonly consumed flat bread. In this investigation, the effects of addition of precipitated whey protein (PWP) and fermentation time on the physical properties and sensory characteristics of Lavash breads were studied. Dough was prepared with four substitution levels of PWP (25, 50, 75 and 100%, instead of water used for dough making) and fermented at three different fermentation times (30, 60 and 90 min). Increasing level of supplementation with PWP generally caused an increase in all sensory scores of Lavash samples. This improvement was more obvious when fermentation time was increased. Penetrometer values of Lavash samples showed that increasing fermentation time and supplementation up to 75% created significantly softer breads compare to control. In general, increase in the level of supplementation and fermentation time significantly increased bread yellowness ( $b^*$  value) and redness ( $a^*$ ) and decreased lightness ( $L^*$  value).

**Key words:** Flat bread, Lavash, precipitated whey protein, fermentation.

## INTRODUCTION

Cereals in general, and wheat, in particular, are principal foods around the world that provide more nutrients than any other single food source. Human beings mastered the use of wheat and the art of bread making thousands of years ago. Nowadays, different breads are produced which can be divided into three categories with respect to their specific volume (volume/weight): those with high specific volume such as pan breads, those with medium specific volume such as French and rye breads, and those with low specific volume such as flat breads (Faridi, 1988).

Flat bread can be divided into two major groups according to their cross section: single-layered and double-layered. The most important processing difference between these two kinds of bread is the second proofing period which might exceed 30 min in double-layered types. Flat breads differ in the type of flour, manner of sheeting or in the appearance of the end product. These products have many characteristics that even appeal to the U.S population (Qarooni et al., 1992).

Flat breads are a major form of wheat consumption in many Middle Eastern and North African countries (Paulley, 1998). Faridy (1981) demonstrated several rea-

sons for the increased popularity of flat breads. First, these products are good source of dietary fiber because flours of high extraction rate, usually more than 78%, generally are used. Second, the formulas are simple, and there are few ingredients. Third, they are excellent food carriers either by incorporating foods such as meat or vegetable in the dough or as a final product in which food can be placed in the pocket of pita or rolled by the single-layered flat breads. And fourth, flat bread production offers some real advantages for the baking industry. The ingredients are few, so there is a significant reduction in cost and storage space. The equipment requirements are relatively few compared to those of pan bread production. Because flat breads are produced directly on the conveyor belts, substantial savings are recognized by eliminating the need for baking trays and pans. Flat breads require less cooling time and space because of their large surface areas. And finally, a large number of products can be made with only slight modifications to an existing production line.

Today in Iran, many types of flat breads, such as Lavash, Sangak and Barbari are produced and consumed. Lavash bread is single-layered bread leavened with sour dough

**Table 1.** Composition (%) of wheat flour and PWP used for Lavash production.

	Moisture	Protein	Fat	Carbohydrate	Ash
Flour	11.94	10.42	1.45	75.71	0.48
PWP	89.06	4.22	1.73	2.41	0.58

/yeast or chemically with sodium bicarbonate.

In recent years, many researchers have tried to improve baking properties, organoleptic characteristics, nutritional value and extension of the shelf-life of loaf bread. Whey and whey products are considered as appropriate ingredients for promoting nutritive value and organoleptic characteristics of many food products including bread and other bakery products (Goecmen, 1993; Riera-Rodriguez, 2002; Drokani et al., 2003; Jooyandeh, 2006; Rantamaki, 2006). Once considered a waste product in the cheese manufacturing process, whey and whey protein products today are used for a wide range of functional and nutritional properties (Hoch, 1997). Whey proteins are an important functional component in bread formulations. They enhance crust browning, crumb structure and flavor, improve toasting qualities and retard staling. Whey-based ingredients can be customized to meet specific protein, minerals and lactose compositions. This is important because composition and degree of denaturation affect whey ingredient functionality (Burrington, 2004).

According to literature, there are scarcely any reports concerning the utilization of PWP in flat bread making. In previous work (Jooyandeh, 2006) different ingredients including whey powder, soy flour, and potato powder at different level of supplementation were used in Lavash production to improve its quality. In this study the effect of using PWP and fermentation time on the quality of Iranian Lavash bread is evaluated.

## MATERIALS AND METHODS

### Preparation of PWP

PWP was prepared from sweet Feta cheese whey by heat treatment and fermentation to coagulate and precipitate whey proteins (Jooyandeh et al., 2009). After collection of whey during preparation of Feta cheese, the whey was heated at 85°C for 15 min. This heat treatment caused denaturation and coagulation which was required for proper precipitation of whey proteins during fermentation. Then, the whey was cooled to 44°C and inoculated with 1 - 2% of yoghurt starter cultures (YO-MIX 532, Danisco, Germany) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. After about 4 h fermentation at 42°C, the pH of fermented whey reduced to 4.4 and fermented whey protein was precipitate as a separate phase. The upper phase was pouring out and remaining part was used as PWP.

### Preparation of Lavash bread

Wheat flour (*Triticum aestivum*) with 84.1% extraction rate was obtained from a local supplier. Lavash bread samples were produced

by a technique involving double rising. In this study, PWP were incorporated in Lavash breads at four substitution levels 25, 50, 75, and 100% instead of water during dough making. Lavash bread samples without these ingredients were prepared and considered as control. The formula used in the preparation of experimental breads was wheat flour, 0.5% dried yeast, 1% salt, and water (optimum as assessed by dough feel at sheeting) or mixture of water and PWP. For all the recipes, the yeast was pre-fermented by suspending it in a sugar solution for 30 min for revitalization.

For standard processing, ingredients were mixed with simultaneous addition of water or/and whey ingredients at a low disk speed (44 rpm for 1 min) and then at higher disk speed (130 rpm for 7 min) in a Hunt 30 dough mixer (Lancashire, UK) until a cohesive dough mass was obtained. The amounts of water needed for optimum dough consistency are shown in Table 1. The resultant bulk dough was fermented at different fermentation times 30, 60 and 90 min in an incubator and maintained at 30°C at 85% relative humidity. The fermented dough was divided into balls of 200 g each. The dough pieces were dusted with flour of the same formulation (~10 g) and rounded into ball shape. The balls were covered with a wet cloth and fermented for 15 min at 30°C and then flattened into elliptical sheets 1.7 mm thickness and baked in commercial automated oven at 470°C to optimum crust color (~50 - 70 s), as reported by Maleki and Dagher (1967). After baking, Lavash breads were cooled for 15 min, placed in polyethylene bags to prevent moisture loss, and stored at room temperature (30°C). All baking parameter and sensory evaluation were measured 2 h after baking.

### Chemical analysis

Protein contents of flour (N × 6.25) and PWP (N × 6.38) were determined by a nitrogen analyzer (Leco Corporation, St. Joseph, MI). Moisture content of flour was determined by oven drying for 1 h at 130°C using AACC (2000) by approved method of 44-15A, ash content by dry combustion for 16 h at 580°C according to approved method 08-01, and fat content by petroleum ether extraction followed by evaporation to constant weight under vacuum using approved method 30-25. Total carbohydrate content (%) was calculated by difference. For PWP, moisture content was determined at 102°C for 4 - 5 h according to AOAC (2000) by official method 925.23, ash using official method 945.46 (at 550°C), and fat by Gerber's method.

### Texture analysis

Sur PNR-6 penetrometer with 218 g of total test weight was used for determine hardness of breads as indicated by Basman and Koksel (1999)

### Color measurement

Crust color was measured with a Minolta Chroma Meter (CR-300, Minolta, Osaka, Japan). This defines colour numerically in terms of lightness or L\* value, (0 = black, 100 = white), a\* value (greenness 0 to -100, redness 0 to +100) and b\* value (blueness 0 to -100, yellowness 0 to +100). Penetrometer and crust color values of each Lavash sample were determined at five different points. All measurements obtained with the three Lavash breads from one batch were averaged into one replicate value.

### Sensory evaluation

Twelve panelists (five female, seven male; aged 20-30) who had completed a graduate course in food quality and were familiar with

**CRUST COLOR**

- (5) Creamish yellow color with light brown patches
- (4) Cream color with light brown patches
- (3) Light cream color with light brown patches
- (2) Lighter gray color with pale or dark brown patches
- (1) Gray color with pale or dark brown patches

**CRUMB COLOR**

- (5) Whitish cream color
- (4) Light yellowish cream color
- (3) Yellowish cream color
- (2) Light grayish cream color
- (1) gray color

**EXTERNAL APPEARANCE AND SHAPE**

- (5) Crust is smooth with few blisters and few cracks on the edges
- (4) 75% of crust is smooth with small amount of blisters and cracks on the edges
- (3) 50% of crust is smooth with moderate amount of blisters and cracks on the edges
- (2) 25% of crust is smooth with high levels of blisters and high levels of cracks on the edges
- (1) Crust is not smooth, very high levels of blisters and cracks

**TASTE AND AROMA**

- (5) Characteristic Lavash aroma and taste
- (4) Light smell and taste from additive
- (3) Perceptible smell and taste from additive
- (2) Definite undesirable taste and smell from additive
- (1) very definite unacceptable smell and taste

**MOUTHFEEL AND TEXTURE**

- (5) Very pleasant and easy to chew
- (4) Pleasant and easy to chew
- (3) Slightly sticky when chewing
- (2) Sticky when chewing
- (1) Very sticky and doughy when chewing (waxy texture)

**Which Lavash samples do you consider acceptable?**

**Figure 1.** Ballot sheet for Lavash samples (Qarooni et al., 1987; Williams, 1988; Saxena and Rao, 1996) with some modifications.

Lavash bread were chosen. All panelists were nonsmokers. Instructions were given in full to panelists beforehand. Examination took place in tasting booths under normal white illumination.

Lavash samples supplemented with PWP were evaluated on a scale of 1 - 5 for five quality parameters: crust color, crumb color, external appearance and shape, taste and aroma, and mouth feel and texture. A ballot sheet (Figure 1) was prepared to evaluate sensory attributes of Lavash breads after modifying parameters and scores of various flat breads to Lavash (Qarooni et al., 1987; Williams, 1988; Saxena and Rao, 1996). Consistency of the panel was checked by subjecting data for the indicated attributes from three replicate rating of nine bread samples to principal component analysis (Kwan and Kowalski, 1980; Powers, 1984). The results (data not shown) revealed a cluster of ten assessors indicating agreement in evaluation, and two outliers. Consequently, sensory measurements were conducted with the consistent panel and final judgment was obtained by averaging the scores given only by these panelists.

Samples, selected at random from the different treatments, were removed from polyethylene bags before evaluation. The breads were rated in comparison to regular wheat bread (without PWP).

**Statistical analysis**

All determinations were made in triplicate, and mean values are

presented. The data collected from studies were analyzed using SPSS version 11.5 (SPSS Inc., Chicago, IL). One way analysis of variance was performed for determination of individual differences between 15 treatments and two way analysis was carried out to determine significant differences in each factor. PWP concentration and fermentation time, the whole-plot factors, were applied in a 5 x 3 factorial treatment structure. A probability of <0.05 was used to establish statistical significance for fixed effects and interactions using Duncan's multiple-comparison test.

**RESULTS AND DISCUSSION****Chemical composition**

The chemical composition of wheat flour and PWP are presented in Table 1. PWP had a higher fat and ash, and lower protein than wheat flour. The pH and acidity of PWP were  $4.5 \pm 0.2$  and  $63 \pm 6.2$  °D (0.63 g percent lactic acid), respectively. This fermented product which rich in protein can be used in different product. Kadharmestan et al. (1998) reported with heat denaturation, whey protein becomes insoluble and loses its functionality like emulsifying and foam ability but it can be used as a non-

**Table 2.** The mean sensory scores of Lavash samples supplemented with PWP and different dough fermentation time.

Level of PWP	Fermentation time (min)	Crust color	Crumb color	External appearance and shape	Taste and aroma	Mouth feel and texture	Overall acceptability
0% (Control)	30	3.87 <sup>h</sup>	4.73 <sup>ab</sup>	4.27 <sup>de</sup>	4.00 <sup>h</sup>	4.07 <sup>e</sup>	4.18 <sup>h</sup>
	60	4.20 <sup>tg</sup>	4.53 <sup>bcd</sup>	4.60 <sup>ab</sup>	4.20 <sup>g</sup>	4.30 <sup>d</sup>	4.37 <sup>f</sup>
25% PWP	90	4.37 <sup>ef</sup>	4.63 <sup>abcd</sup>	4.20 <sup>e</sup>	4.40 <sup>ef</sup>	4.50 <sup>c</sup>	4.42 <sup>ef</sup>
	30	4.13 <sup>g</sup>	4.73 <sup>ab</sup>	4.40 <sup>cd</sup>	4.13 <sup>gh</sup>	4.03 <sup>e</sup>	4.28 <sup>g</sup>
	60	4.33 <sup>ef</sup>	4.73 <sup>ab</sup>	4.70 <sup>a</sup>	4.30 <sup>tg</sup>	4.50 <sup>c</sup>	4.51 <sup>d</sup>
50% PWP	90	4.40 <sup>e</sup>	4.63 <sup>abcd</sup>	4.50 <sup>b</sup>	4.60 <sup>cd</sup>	4.60 <sup>bc</sup>	4.57 <sup>d</sup>
	30	4.30 <sup>efg</sup>	4.63 <sup>abcd</sup>	4.60 <sup>ab</sup>	4.20 <sup>g</sup>	4.47 <sup>c</sup>	4.44 <sup>e</sup>
	60	4.57 <sup>cd</sup>	4.83 <sup>a</sup>	4.50 <sup>bc</sup>	4.50 <sup>de</sup>	4.70 <sup>b</sup>	4.62 <sup>c</sup>
	90	4.87 <sup>ab</sup>	4.73 <sup>ab</sup>	4.40 <sup>cd</sup>	4.70 <sup>bc</sup>	4.90 <sup>a</sup>	4.72 <sup>a</sup>
75% PWP	30	5.00 <sup>a</sup>	4.53 <sup>bcd</sup>	4.60 <sup>ab</sup>	4.50 <sup>de</sup>	4.60 <sup>bc</sup>	4.67 <sup>bc</sup>
	60	4.83 <sup>b</sup>	4.63 <sup>abcd</sup>	4.70 <sup>a</sup>	4.60 <sup>cd</sup>	4.90 <sup>a</sup>	4.73 <sup>a</sup>
	90	4.60 <sup>c</sup>	4.43 <sup>ed</sup>	4.23 <sup>de</sup>	4.90 <sup>a</sup>	4.97 <sup>a</sup>	4.67 <sup>c</sup>
100% PWP	30	4.77 <sup>b</sup>	4.63 <sup>abcd</sup>	4.60 <sup>ab</sup>	4.80 <sup>ab</sup>	4.50 <sup>c</sup>	4.69 <sup>ab</sup>
	60	4.43 <sup>de</sup>	4.43 <sup>ed</sup>	4.20 <sup>e</sup>	4.97 <sup>a</sup>	4.70 <sup>b</sup>	4.57 <sup>d</sup>
	90	4.27 <sup>efg</sup>	4.23 <sup>e</sup>	3.90 <sup>f</sup>	4.70 <sup>bc</sup>	4.67 <sup>b</sup>	4.40 <sup>ef</sup>

<sup>a,b,c,d</sup> Means within the same column having different letters are significantly different (p< 0.05).

**Table 3.** Probability values from ANOVA of the effects of different PWP supplementations and fermentation time on sensory properties of Lavash breads.

Source	Fermentation time (min)	Crust color	Crumb color	External appearance and shape	Taste and aroma	Mouth feel and texture	Overall acceptability
PWP	F value	67.538	10.275	16.378	53.191	63.000	154.301
	Significance <sup>1</sup>	0.000	0.000	0.000	0.000	0.000	0.000
Fermentation time	F value	3.325	4.650	40.902	40.085	69.939	48.619
	Significance	0.050	0.017	0.000	0.000	0.000	0.000
PWP × Fermentation time	F value	21.919	2.962	11.421	5.830	5.432	42.919
	Significance	0.014	0.000	0.000	0.000	0.000	0.000

1. Significance at the 0.05 level.

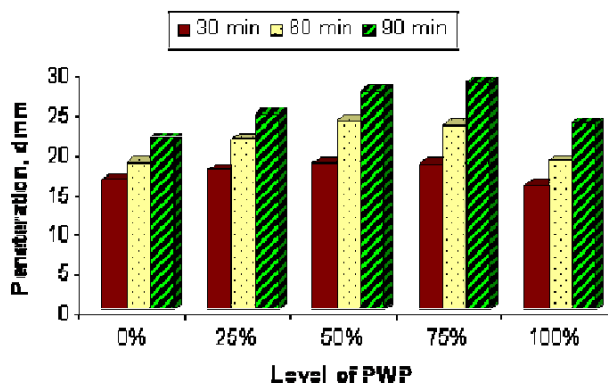
functional ingredient for fortification of baked goods. Many other researchers (Vetter, 1984; Erdogdu-Arnoczky and Pomeranz, 1996; Kenny et al., 2001) also emphasized that whey protein denaturation is compulsory for production of a high quality bread.

**Sensory properties**

The mean sensory scores of Lavash samples supplemented with PWP and different dough fermentation time are presented in Table 2. Results showed that both supplementation and fermentation time have significant effects on all organoleptic characteristics of Lavash breads. There were also significant interactions between incorporation levels and fermentation time in all sensory attri-

butes (Table 3). Supplemented breads had higher acceptability and increase in the supplementation level usually caused an increase in sensory scores. Lavash samples made with 100% incorporation had better taste, but lower appearance and crumb color than other samples, including control. With respect to crust colour and taste, all levels, including control, were classified in different groups. Control Lavash (without PWP) significantly had lower scores than supplemented samples in terms of crust color, taste and aroma, texture and mouth feel, and overall acceptability.

Sensory scores of Lavash samples made with fermented dough at different fermentation times also revealed that increase in fermentation time cause an increase in all sensory parameters. No differences was observed among



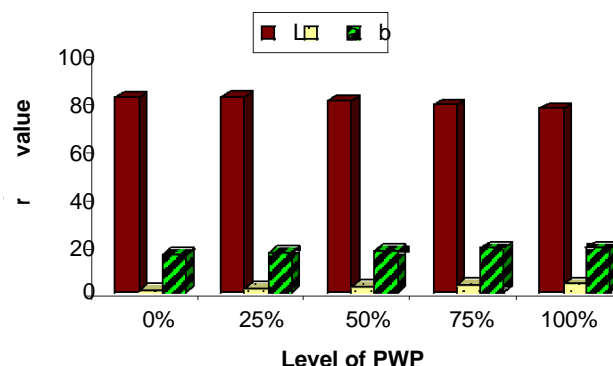
**Figure 2.** Influence of PWP supplementation and fermentation time on penetrometer values of Lavash breads.

60 and 90 min fermentation times on the sensory parameters of Lavash samples except for taste and mouth feel which were higher in samples fermented at 90 min.

The one-way analysis of sensory scores ( $p < 0.05$ ) indicated that the best overall acceptability can be obtained by 50, 75 and 100% supplementations at different fermentation times 90, 60 and 30 min, respectively (Table 2). This proved that with increase the level of supplementation, fermentation time can be considerably reduced.

### Bread texture

Results indicated that supplementation and fermentation time have significant effects on the softness of Lavash samples in different manner (Figure 2). In supplemented Lavash samples, the softness increased up to 50% and thereafter declined. However, all supplemented samples were softer than control and there was no difference between samples fortified with 50 and 75% PWP. The penetration rates for control and supplemented samples were 18.9, 21.2, 23.3, 23.4, and 19.3 respectively. The increase and then decrease in softness might be attributed to the mutual effect of addition of PWP. Supplementation at higher level of PWP decreased softness and increased fermentation rate. The decrease in softness due to higher level of PWP may be related to higher amount of ash and calcium available in PWP. The amount of calcium plays a significant role in the rate of firming of bread (Burrington, 2004). The effect of fermentation time on the softness of Lavash samples was more obvious than that of the level of PWP supplementations. Increase in the fermentation time caused significant increase in Lavash softness. Burrington (2004) expressed that decreasing fermentation time is detrimental to bread quality when whey protein concentrates are used. Typically, the shorter the fermentation time, the more sensitive the bread is to whey proteins. This effect was obviously observed in this study. During sourdough preparation, lactic acid bacteria produce a number of metabolites which have been shown to have a positive effect

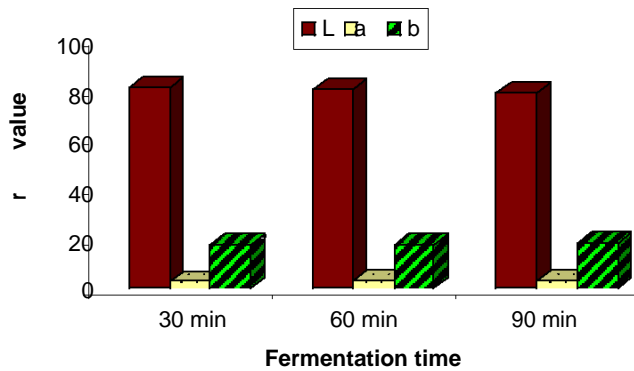


**Figure 3.** Influence of PWP on crust color (L, a, and b values) of Lavash breads.

on the texture and staling of bread, e.g. organic acids, exopolysaccharides and/or enzymes (Arendt, 2007). Organic acids affect the protein and starch fractions of flour. Additionally, the drop in pH associated with acid production causes an increase in the proteases and amylases activity of the flour, thus leading to a softer texture. Samples of Lavash supplemented with 50 and 75% PWP and fermented at 90 min were significantly softer than the rest of the supplementation levels and the control. The penetration rates for these samples were 27.6 and 28.7 dmm, respectively. The penetration rates for Lavash control samples fermented at 30, 60 and 90 min were 16.4, 18.6 and 21.6 dmm, respectively.

### Bread color

A distinctive characteristic of the supplemented samples was their crust color. All samples which contained PWP, particularly at higher amount of incorporation, exhibited a white-yellowish color and differences among the samples were already visible without instrument aid. Supplementation with higher amount of PWP and increase in the fermentation time significantly increased bread yellowness ( $b^*$  value) and redness ( $a^*$ ) in the Lavash samples; whereas lightness ( $L^*$ ) adversely was affected by these factors (Figure 3 and 4). Results showed that the crust color changes were significantly higher in samples contain 75 and 100% substitutions than others ( $p = 0.003$  and  $p = 0.000$ ; respectively). The reason can be explained by Maillard reaction. The Maillard reaction is quite universal in the food industry, and this reaction occurs when most foods are heated and results in reactions that promote browning of cookies, bread, and other baked goods. Whey proteins contain a high amount of the amino acid lysine (Trierum, 2004) which is typically the most reactive amino acid in regard to the Maillard reaction because it possesses the  $\alpha$ -amino group (deMan, 1999). On the other hand, reducing sugars which are another important factor in reaction are present in PWP. Therefore, increase in PWP incorporation in Lavashes led to increase in Maillard reac-



**Figure 4.** Influence of fermentation time on crust color (L, a, and b values) of Lavash breads.

tion.

The effect of level of PWP supplementation on the crust color of Lavash samples was more obvious than the effect of different fermentation times. However, both these factors created significant differences on crust color. In Lavash samples, as the percent of PWP incorporation or fermentation time increased, L values shifted from white to gray, a values shifted from gray to red, and b values shifted from gray to yellow, significantly. The L value for control (80.7) was higher than samples prepared with 50, 75, and 100% PWP (79.5, 77.7, and 76.3, respectively) and lower than sample with 25% PWP (81.1). However, there was not significant difference between control and samples contain 25 and 50% PWP. The a and b value of Lavash samples varied from 0.9 to 3.6 and 15.8 to 18.4 respectively; the highest and lowest were related to control and samples supplemented with 100% PWP, respectively.

## Conclusion

Supplementation with PWP generally led to quality improvement of Lavash breads and softer bread. Increasing the level of PWP was significantly increased penetration rates and it ranged from 18.9 (control) to 23.4 (75% PWP). However, at the highest level of supplementation, that is, breads with 100% PWP, the penetration rate was noticeably reduced and reached to 19.3 dmm. On the other hand, with increasing the fermentation time, the softer bread with higher sensory scores was produced. The penetration rates for breads fermented at 30, 60 and 90 min were 17.3, 21.1 and 25.2 dmm; respectively. In summarize, samples contained 50 or 75 % PWP and fermented for 90 min had better quality. It was also obviously observed that by increasing the rate of supplementation and decrease in the fermentation time, Lavash bread with a good quality can be produced. The results indicated that the process time and preconditioning cost can be considerably lowered by adopting the approach

suggested in this paper, while the quality of Lavash breads is improved.

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