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Effects of dough improvers on micro-structural, textural, rheological, and baking properties of frozen dough with virgin coconuts oil

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Abstract

This research aimed to investigate effects of dough improvers (trehalose; TR, sucrose ester; SE and hydroxylpropylmethylcellulose; HPMC) on microstructural, textural, rheological and baking properties of frozen dough with virgin coconut oil using central composite design (CCD). Frozen dough containing dough improvers had higher values of all properties compared to frozen dough without dough improvers ($p \leq 0.05$). All properties of the highest accepted dough; 8% TR, 1.5% SE and 1.5% HPMC and virgin coconut oil were 7.79 score, 25.59 min, 7.65 mg/g, 2814.14 g-force, 40.12 Pa.s, and 23.68 Pa.s as represented overall acceptance, mixing stability, specific volume, hardness, G' and G", respectively. SEM and CLSM images of this highest accepted dough exhibited evenly distributed fat phase, and smaller and smoother gluten structure. This work suggested that TR, SE, and HPMC were alternative dough improvers for frozen bread dough with virgin coconut oil.

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Introduction

The overall quality of frozen dough bread deteriorates gradually during frozen storage (Kenny *et al*., 2001). The Loss of bread quality have been identified as possible reasons including a decline in both viability and yeast activity lead to reduce in gassing power and the gradual decrease in dough strength (Ribotta *et al*., 2004). During frozen storage, gradual loss of dough strength has been attributed to decrease gluten cross-linking caused by ice recrystallization and redistribution of the water provoked by a modification in the water binding capacity of dough constituents (Ribotta *et al*., 2003a). Improvement of frozen bread dough quality used dough improver has been studied.

Dough improvers, including emulsifiers, hydrocolloids and other improvers have been used to improve the quality parameters of fresh and stored baked products (Selomulyo and Zhou, 2007). Trehalose (TR;α-D-glucopyranosyl [1–1]-α-D-glucopyranoside), a major storage carbohydrate in Saccharomyces cerevisiae yeast, has been found to protect biomolecules against environmental stress caused by desiccation, dehydration, heat, freezing, and oxidation (Elbein *et al*., 2003; Gancedo and Flores 2004). Many researchers studied that addition of TR in dough formulations improved quality of bread (Kim *et al*., 2008; Huang *et al*., 2008). Sucrose ester (SE) emulsifier was added in dough formulations to improve bread quality such as fine and soft crumb structure, high volume, extended shelf life, increased dough mixing tolerance, and improved freeze–thaw stability (Barrett *et al*., 2002). Moreover, Hosomi *et al*. (1992) found that addition of SE prevented wheat protein denaturation during freezing. Selomulyo and Zhou (2007) reported that SE improved sensory properties of bread including firmness, cohesiveness, chewiness, and moistness. Hydroxypropylmethylcellulose (HPMC) hydrocolloids revealed the etherification of hydroxyl groups of the cellulose by methoxyl and hydroxypropyl groups. HPMC was added in dough formulations to increase its water solubility and also confers some affinity for the non-polar phase in doughs, in a multiphase system like bread dough (Selomulyo and Zhou, 2007). The addition of HPMC in dough formulation improved specific volume and crumb texture of bread (Rosell *et al*., 2001) and increased the shelf life of partially baked bread (Barcenas and Rosell, 2006). Moreover, HPMC had proved the most effective in structuring baked products, which forms

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thermoreversible gel networks on heating (Sabanis and Tzia, 2011). However, optimal formulations used TR, SE and HPMC for improving frozen bread dough quality, has not been systematically determined.

Therefore, the objectives of this study was to investigate the combine effect of three ingredients, TR, SE and HPMC, in bread dough formulation with virgin coconut oil, on mixing stability, loaf specific volume, crumb firmness, rheological property and overall acceptability using a response surface methodology. Microstructures of frozen bread dough have been studied.

Material and Methods

Materials

A commercial bread flour with moisture, protein, fat, and ash contents of 14%, 13.03%, ,0.78%, and 0.57 (Approved methods 40-15, 08-01, 46-13, and 30-25, respectively) (AACC, 1995) was purchased from United Flour Mill Public Co., Ltd. (Bangkok, Thailand). Optimum amount of water (OAW) of 62.0%, mixing stability of 26.5% and flour dough properties were evaluated using a farinograph (Kulturstrasse 51-55 D-47055, Brabender® GmbH & Co. KG, Duisburg, Germany) (AACC, 1995). Trehalose (TR) and hydroxpropylmethyl cellulose (HPMC) were purchased from Sigma Chemical Company (AR grade, St. Louis, Mo, USA). Sucrose ester (SE) was obtained from Mitsubishi-Kagaku Foods (RYOTO®, AR grade, Mitsubishi-Kagaku Foods Co., Japan). Virgin coconut oil was provided from Natural mind co., Ltd. (Chumporn, Thailand). Shortening was obtained from Three Top co., Ltd. (Bangkok, Thailand). All other chemicals were of analytical grade.

Dough preparation and frozen dough

Dough ingredients consist of flour, water (60% flour weigth basis, fwb), instant dry yeast (1.5% fwb), salt (1.5% fwb), shortening or virgin coconut oil (5% fwb) and sugar (4% fwb). A no-time-dough procedure was used. The amounts of TR, SE, and HPMC were formulated according to the experimental design using central composite design (CCD) method with the modified Kim *et al*. (2008) in Table 1. Optimum amount of water (OAW) used to calculate amount of water (60% fwb) which it was required by a farinograph minus 2.6% points as typical frozen dough uses 2-3% points less than the farinograph optimum water absorption (Kim *et al*., 2008). OWA was evaluated combining flour with TR, SE, and HPMC at the center level of experimental design (Table 1), 6%, 1%, and 1% (fwb), respectively.

The dough was mixed until gluten was optimally developed for 8 min at 25±1°C. After 10 min waiting for yeast activity, 280 g of dough was wrapped with a polyethylene sheet and stored in an air blast freezer at −35°C until its core temperature was at −20°C. Subsequently, dough was stored at −18°C for 3 months in a freezer. Dough was thawed at room temperature until its core temperature was at 25°C. Then, dough was immediately proofed at 35°C (90% relative humidity) and baked at 200 °C for 20 min after molding (non-frozen dough). All samples were cooled at room temperature for 1 h and kept in plastic bag until analysis. All frozen dough and breads were determined the structural, textural, rheological and baking properties.

Loaf volume

Loaf volume and weight were measured 1 h after baking using a sesame seed displacement method and specific volume was obtained from loaf volume divided by weight (Sangnark and Noomhorm, 2003).

Hardness

All samples were determined by following the method of Stable Micro System Ltd. (1995) using a TA-XT2 (Stable Microsystems, Godalming, UK) equipped with a cylindrical P/35 probe after 24 h of baking. Four bread slices were compressed to 25% of their original thickness. The compression rate was set at 1.0 mm/s. The maximum force read from the highest positive peak was used an indicator of the hardness of sample. Data were reported as the mean of twelve measurements, which were performed on three loaves from different experiments.

Dynamic rheological measurements

Rheological property of dough was determined by small-amplitude oscillatory rheological test using rheometer (1000-N, TA Instruments, Newcastle, DE, USA) equipped with a parallel-plate sensor (517400.901 C/H, 4 cm diameter, 1 mm gap). The sample was thawed at room temperature until its core temperature was at 25°C. Subsequently, the sample was placed on the lower plate and excess dough protruding from the edge of the plate was carefully trimmed. Low viscosity silicone was added around the plate edges to prevent dough dehydration. Temperature was kept constant at 25°C. Selection of strain was optimum in Linear Viscoelastic Region (LVR).

Frequency Sweep Test of frozen dough was also determined using rheometer. Sample was rested for 2 min after loading before testing. This resting time was sufficient for the dough to relax. Data obtained were storage modulus (G′) and loss modulus (G″)

Table 1. The experimental design of independent variables and levels used in the preparation of frozen dough bread using central composite design (CCD) method.

Treatment	TR[b]	SE[c]	HPMC[d]	Fat
1	4	0.5	0.5	Virgin coconut oil
2	8	0.5	0.5	Virgin coconut oil
3	4	0.5	1.5	Virgin coconut oil
4	8	0.5	1.5	Virgin coconut oil
5	4	1.5	0.5	Virgin coconut oil
6	8	1.5	0.5	Virgin coconut oil
7	4	1.5	1.5	Virgin coconut oil
8	8	1.5	1.5	Virgin coconut oil
9	2.64	0.5	0.5	Virgin coconut oil
10	9.36	0.5	0.5	Virgin coconut oil
11	6	0.5	0.16	Virgin coconut oil
12	6	0.5	1.84	Virgin coconut oil
13	6	0.16	1	Virgin coconut oil
14	6	1.84	1	Virgin coconut oil
15	6	1	1	Virgin coconut oil
16	6	1	1	Virgin coconut oil
17	6	1	1	Virgin coconut oil
18	6	1	1	Virgin coconut oil
19	6	1	1	Virgin coconut oil
20	6	1	1	Virgin coconut oil
*21	NO	NO	NO	Virgin coconut oil
*22	NO	NO	NO	Shortening

[a]Based on flour weight basis (100%)
[b]Trehalose
[c]Sucrose ester
[d]hydroxpropylmethyl cellulose
[e]α= -1.68
[f]α= 1.68
*Treatment 21 and 22 were control.

as function of time and frequency of oscillation. Deformation of 3% was determined within the linear viscoelasticity range with a modification method from Rosell and Foegeding (2007). Three replicates were analysed and results were expressed as mean values±SD.

Sensory evaluation

Breads were evaluated on the basis of acceptability of their flavor, volume, softness, and overall acceptance. A 9-point hedonic scale was used. A score of 1 represented ‘dislike extremely’ and a score of nine represented ‘like extremely’. The 50 panelists were Food Science students and ranged in age from 22 to 25 years. Samples were randomly coded and served individually.

Scanning electron microscopy (SEM)

Morphology of frozen dough was evaluated by scanning electron microscope (SEM) (JSM – 6400, LV, Jeol, Japan) with a modification from Ribotta *et al*. (2004) method. Small portions of stored dough frozen at −18±2°C for 1 and 90 days were cut with a razor blade. All samples were thawed and fixed in glutaraldehyde (1:30) for 2 h and embedded in a graded acetone series (25, 50, 75 and 80%) for 20 min at each gradation. Finally, samples embedded in 100% acetone at three consecutive 20 min intervals to ensure full dehydration and then were critical point dried. Critical point drying allows acetone removal in CO_2 without surface tension force that may distort the sample. Dehydrated samples were coated with 4 µm gold using sputter coated (Ion Sputtering Device JFC – 110E, Japan), examined and photographed at an accelerating voltage of 6 kv with a magnification of 10,000×.

Confocal scanning laser microscopy (CSLM)

Frozen dough microstructure was visualised using confocal scanning laser microscopy according to the procedure described by Nagano et al. (2007) with a modification method (LEICA TCS NT microscope, Leica Microsystems, Heildelberg, Germany). 0.5 g of dough was stained with fluorescent dye and kept in dark room at 4°C for 2 h. Stained frozen doughs were defrosted at ambient temperature at least 1 h before CSLM observations. In the stained samples, fat was red while the protein and starch granule were grey.

Experimental design and statistical analysis

All experiments were performed in a central composite design (CCD) with 3 factors at 3 levels (Table 1). The factors included levels of TR (X1), SE (X2), and HPMC (X3). Following the design principles of a uniform precision, the central point was repeated 6 times in treatment 15–20 and control in treatment 21-22 (Table 1). To assure the rotatability in CCD, α was chosen as 1.68. Analysis of variance (ANOVA) and Duncan’s multiple range tests was used to determine differences among treatments (Steel and Torrie, 1980). The statistical analyses were performed using SPSS Statistics Program (SPSS 12.0 for windows, SPSS Inc., Chicago, IL). Significance of differences was defined at $p \leq 0.05$.

Results and Discussion

Mixing properties

The farinograph results of all samples are shown in Table 2. All sample decreased mixing stability comparing with commercial flour of 26.5 min. Frozen dough containing virgin coconut oil (without dough improvers) exhibited the lowest mixing stability of 15.54 min, while frozen dough with dough improver including of treatment 4 (8% TR, 0.5% SE, 1.5% HPMC), 8 (8% TR, 1.5% SE, 1.5% HPMC) and 12 (6% TR, 0.5% SE, 1.84% HPMC), exhibited the highest mixing stability range of 24.28-25.29 min ($p \leq 0.05$). These results indicated that dough improvers may have a potential to replace a portion of wheat flour without causing detrimental consequences for dough quality. Similar effect on dough strength was observed by Kim *et al*. (2008) when TR was added to wheat flours. In addition, TR, SE and HPMC supplement in dough formulations to produce bread increased dough mixing tolerance (Barrett *et al*., 2002; Selomulyo and Zhou, 2007;

Table 2. Mixing stability, specific volume, textural and rheological properties of frozen dough and bread

Treatment	Mixing stability (min)	Specific volume (mg/g)	Hardness (g force)	G' (Pa.s)	G" (Pa.s)
1	20.49^{e} ± 2.35	± 0.98	± 52.17	± 3.18	18.21^{d} ± 2.12
2	21.25^{de} ± 2.59	7.16^{ef} ± 2.65	2928.82^{e} ± 51.12	21.26^{g} ± 2.16	11.27^{e} ± 2.24
3	23.39^{bc} ± 3.17	7.33^{cd} ± 2.17	3160.12^{d} ± 61.98	38.12^{ab} ± 6.21	21.22^{bc} ± 3.13
4	24.28^{ab} ± 2.21	7.50^{ab} ± 1.25	3190.46^{d} ± 63.22	39.24^{a} ± 4.25	21.48^{b} ± 2.19
5	21.52^{de} ± 4.14	7.24^{de} ± 2.18	2814.12^{i} ± 56.13	35.21^{abc} ± 5.13	20.85^{bc} ± 2.26
6	22.39^{cd} ± 3.13	7.46^{cd} ± 1.15	2830.45^{j} ± 59.64	34.23^{cd} ± 3.12	20.42^{bc} ± 4.21
7	23.46^{bc} ± 2.18	7.54^{ab} ± 2.61	2912.23^{g} ± 52.11	37.25^{abc} ± 6.17	21.56^{bc} ± 3.12
8	25.59^{a} ± 4.21	7.65^{a} ± 2.28	2814.14^{i} ± 61.27	40.12^{a} ± 8.23	23.68^{a} ± 4.15
9	20.56^{e} ± 2.19	± 1.65	3018.12^{c} ± 72.21	30.14^{e} ± 6.26	18.22^{d} ± 2.25
10	21.23^{cd} ± 4.16	7.23^{de} ± 2.84	2906.12^{h} ± 65.14	33.22^{d} ± 4.28	20.32^{d} ± 4.22
11	20.35^{e} ± 3.14	7.15^{ef} ± 1.35	3016.23^{c} ± 58.11	29.25^{e} ± 3.21	18.26^{d} ± 2.17
12	25.32^{a} ± 2.25	7.56^{ab} ± 1.69	2939.12^{h} ± 62.10	40.21^{a} ± 5.12	23.42^{a} ± 3.18
13	22.21^{cd} ± 2.14	7.29^{de} ± 2.26	2806.21^{j} ± 53.25	34.26^{cd} ± 4.15	20.45^{bc} ± 5.21
14	23.36^{bc} ± 4.25	7.44^{bc} ± 2.48	2901.62^{h} ± 65.16	37.24^{abc} ± 6.19	21.12^{bc} ± 2.11
15	22.56^{bcd} ± 2.21	7.39^{cd} ± 1.18	2906.12^{h} ± 62.11	35.12^{bcd} ± 3.12	20.81^{bc} ± 4.24
16	22.52^{bcd} ± 3.15	7.32^{cd} ± 2.24	2904.62^{h} ± 59.13	35.24^{bcd} ± 7.21	20.88^{bc} ± 3.12
17	23.45^{bcd} ± 2.14	7.36^{cd} ± 2.74	2912.18^{g} ± 69.39	35.54^{cd} ± 6.25	20.85^{bc} ± 4.25
18	22.53^{bcd} ± 2.24	7.31^{cd} ± 2.12	2902.18^{h} ± 68.14	35.43^{bcd} ± 6.26	20.81^{bc} ± 3.23
19	23.36^{bcd} ± 2.39	7.33^{cd} ± 0.64	2908.23^{h} ± 67.24	35.24^{bcd} ± 4.21	20.88^{bc} ± 3.11
20	22.58^{bcd} ± 2.22	7.37^{cd} ± 0.39	2912.43^{g} ± 73.21	35.48^{cd} ± 5.11	20.85^{bc} ± 2.13
*21	15.54^{g} ± 3.13	5.95^{h} ± 0.64	3541.45^{a} ± 81.18	11.34^{i} ± 2.15	± 2.19
*22	± 4.24	6.10^{g} ± 1.61	3264.42^{b} ± 75.19	15.23^{h} ± 2.11	±1.59

*Treatment 21 and 22 were control.
Different superscripts in the same column indicate statistical difference (p≤0.05).
Data are mean of triplicates.

Table 3. Sensory test of bread obtained from frozen dough stored for 90 days

Treatment	Flavor	Softness	Volume	Overall acceptance
1	7.65^{a} ± 1.58	7.23^{a} ± 1.21	6.90^{ab} ± 1.32	7.25^{b} ± 1.45
2	7.89^{a} ± 2.21	7.15^{a} ± 1.30	6.92^{ab} ± 1.30	7.38^{b} ± 1.43
3	7.86^{a} ± 2.36	7.26^{a} ± 1.14	7.27^{ab} ± 1.45	7.21^{b} ± 1.21
4	7.65^{a} ± 1.24	7.18^{a} ± 1.21	7.17^{ab} ± 1.21	7.35^{b} ± 1.12
5	7.60^{a} ± 1.30	7.29^{a} ± 2.20	7.10^{ab} ± 1.12	7.48^{b} ± 1.21
6	7.85^{a} ± 1.67	7.34^{a} ± 2.33	7.02^{ab} ± 1.07	7.25^{b} ± 1.54
7	7.80^{a} ± 1.34	7.27^{a} ± 2.17	7.30^{a} ± 1.31	7.52^{b} ± 2.21
8	7.98^{a} ± 2.34	7.36^{a} ± 2.31	7.35^{a} ± 1.10	7.79^{a} ± 1.31
9	7.62^{a} ± 1.24	7.19^{a} ± 1.23	7.23^{ab} ± 1.40	7.20^{b} ± 1.54
10	7.80^{a} ± 2.32	7.27^{a} ± 1.30	7.31^{ab} ± 1.21	7.45^{b} ± 2.32
11	7.75^{a} ± 1.56	7.14^{a} ± 1.20	6.94^{ab} ± 1.05	7.30^{b} ± 1.21
12	7.64^{a} ± 1.52	7.18^{a} ± 1.21	7.38^{a} ± 2.23	7.10^{bc} ± 1.52
13	7.68^{a} ± 1.39	7.16^{a} ± 1.03	7.04^{ab} ± 1.08	7.22^{b} ± 1.65
14	7.72^{a} ±1.17	7.32^{a} ± 2.04	7.18^{ab} ± 1.21	7.60^{a} ± 1.48
15	7.68^{a} ± 1.32	7.37^{a} ± 2.42	7.13^{ab} ± 1.28	7.28^{b} ± 1.34
16	7.67^{a} ±1.22	7.33^{a} ± 2.28	7.23^{ab} ± 1.12	7.26^{b} ± 1.34
17	7.87^{a} ± 2.11	7.33^{a} ± 2.15	7.14^{ab} ± 1.20	7.30^{b} ± 1.23
18	7.80^{a} ± 1.23	7.31^{a} ± 1.21	7.16^{ab} ± 1.31	7.27^{b} ± 1.38
19	7.78^{a} ± 1.23	7.29^{a} ± 1.21	7.24^{ab} ± 1.21	7.40^{b} ± 1.54
20	7.73^{a} ± 1.94	7.39^{a} ± 2.41	7.20^{ab} ± 1.13	7.46^{b} ± 1.43
21	7.65^{a} ± 1.12	6.13^{c} ± 1.33	6.70^{b} ± 0.21	6.92^{bc} ± 1.38
22	6.79^{b} ± 0.54	6.54^{b} ± 0.21	6.20^{c} ± 1.54	6.63^{c} ± 1.22

Different superscripts in the same column indicate statistical difference (p≤0.05), n=20.

Huang *et al*., 2008).

Specific volume

The effect of dough improvers (TR, SE, and HPMC), virgin coconut oil and shortening on specific volume is shown in Table 2. The specific volume of frozen dough with virgin coconut oil and shortening exhibited the lower than dough improver (p≤0.05). The highest specific volume exhibited frozen dough containing dough improver of treatment 4 (8% TR, 0.5% SE, 1.5% HPMC), 7 (4% TR, 1.5% SE, 1.5% HPMC), 8 (8% TR, 1.5% SE, 1.5% HPMC) and 12 (6% TR, 0.5% SE, 1.84% HPMC) range of 7.50-7.65 mg/g (p≤0.05). It should be noted that a dough improver helping the restructuring the damaged gluten network is needed in frozen dough system. These results agreement with Barrett *et al*. (2002) and Barcenas *et al*. (2004) reported that frozen dough containing HPMC and SE showed high specific volume. Sarkar and Walker (1995) reported that HPMC forms gels by interacting with the hydrocolloid chains creating a temporary network when the temperature rises during baking, leading to some strength of the dough during expansion and to protect against volume loss. However, Huang *et al*. (2008) found that the addition of the exogenous TR in frozen dough did not significantly improve specific volume of bread.

Hardness

Hardness of frozen dough bread with virgin coconut oil and shortening exhibited the higher than dough improver (p≤0.05, Table 2). Dough formulation with dough improver of treatment 6 (8% TR, 1.5% SE, 0.5% HPMC) and 13 (6% TR, 1.6% SE, 1.0% HPMC) showed the lowest hardness range of 2806.21-2830.45 g-force (p≤0.05, Table 2). It should be note that bread quality on textural properties especially hardness is partially related to the specific volume of bread, larger the specific volume, softer the bread (Basman *et al*., 2002). The addition of SE to produce bread exhibited soft crumb structur (Barrett *et al*., 2002). Moreover, the effect of HPMC on the crumb softness in bread has been attributed to a number of mechanisms including interactions with protein that serves to modify the gluten structure (Grosskreutz, 1961; Krog, 1981). HPMC forms gels also acts as a barrier against a decrease in moisture content, leading it to provide better texture and softness without conferring any adverse effect on the palatability of the bread (Selomulyo and Zhou, 2007).

Dynamic rheological properties of frozen dough

Rheological patterns of all frozen dough exhibited the storage modulus (G′) higher than the loss modulus (G″) in the entire range of frequency (p≤0.05, Table 2). G′ and G″ value of frozen dough with virgin coconut oil and shortening showed the lower than dough improver of all treatment (p≤0.05). These results indicated that addition of dough improver in frozen dough with virgin coconut oil protected the reduction of dough firmness and elasticity cause a loss in the polymer cross-linking and depolymerization during freezing and frozen storage. Reduction of gluten cross-linking was caused by ice recrystallization and/or by

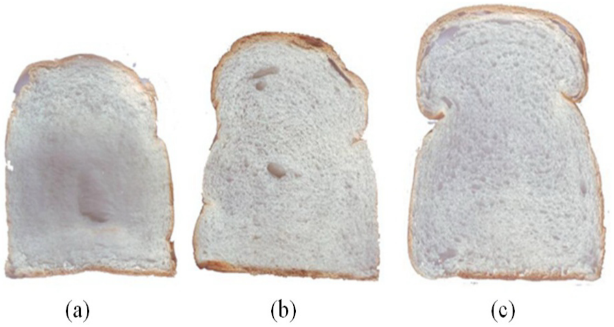

Figure 1. Specific volume of bread obtained from frozen dough stored various formulation at -20°C for 1 day; (a) virgin coconut oil, (b) shortening , and (c) 8% TR, 1.5% SE, 1.5% HPMC

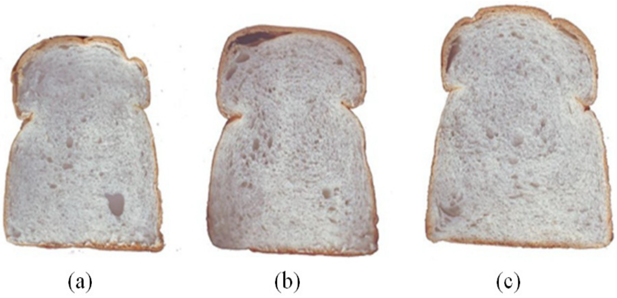

Figure 2. Specific volume of bread obtained from frozen dough stored various formulation at -20°C for 90 day; (a) virgin coconut oil, (b) shortening, and (c) 8% TR, 1.5% SE, 1.5% HPMC

release of low substances from yeast (Ribotta *et al*., 2001). These results agreed with Hosomi *et al*. (1992) who found that addition of sugar ester improved the baking and rheological properties of frozen dough and decreased yeast damage by increasing the amount of non-frozen water in the wheat starch, which acts as a cryoprotectant for yeast cells. Moreover, Salas-Mellado and Chang (2003) reported that exogenous TR also has been applied to protect the yeast during freezing in bread dough system.

Sensory evaluation

Sensory evaluation was performed on bread obtained from frozen dough stored for 90 days (Table 3). Flavor, volume and softness acceptance of bread with shortening exhibited lower than bread containing dough improver with virgin coconut oil ($p \leq 0.05$). Overall acceptance of bread containing dough improver with virgin coconut oil exhibited highest of the treatments 8 (8% TR, 1.5% SE, 1.5% HPMC) and 14 (6% TR, 1.84% SE, 1.0% HPMC) of 7.60-7.79 score ($p \leq 0.05$). Consideration of production costs and all properties lead to select the addition of dough improvers in bread with virgin coconut oil products was treatments 8 (Figures 1 and 2).

Microstructure of frozen dough bread

SEM was used to visualize the ultra structure of frozen dough with virgin coconut oil, shortening and dough improvers and virgin coconut oil stored

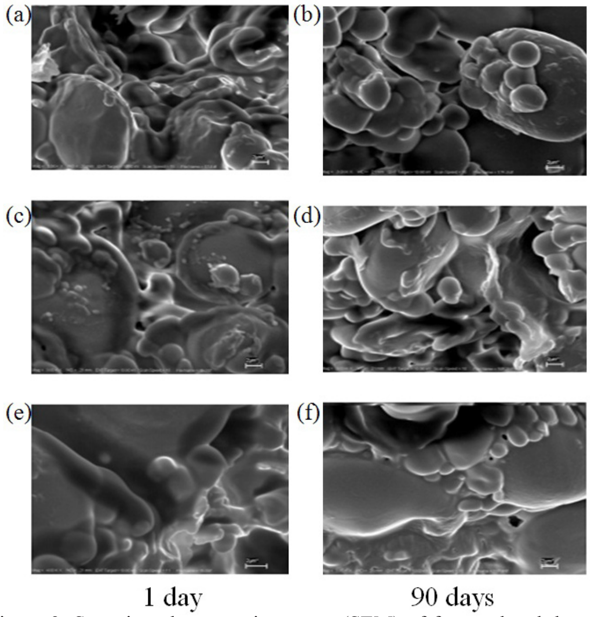

Figure 3. Scanning electron microscope (SEM) of frozen dough bread at1000× for 1 and 90 days; (a-b) virgin coconut oil, (c-d) shortening stored, and (e-f) 8% TR, 1.5% SE, 1.5% HPMC and virgin coconut oil

at -20°C for 1 and 90 days (Figures 3a-3f). Under 1,000× magnification, frozen dough structure of all sample stored for 1 day showed the characteristic structure of the starch granules embedded in the gluten network (Figures 3a, 3c, 3f). After 90 days storage time at −20°C, gluten matrixes of frozen dough with virgin coconut oil and shortening were found to be quite damaged. The gluten strands appeared more porous, less uniform and thinner. Besides, frozen dough tended to break into small pieces due to the disruption of the strand gluten. While the structures of all frozen doughs with dough improvers were not different from those of frozen doughs stored for 1 day (Figures 3b, 3d, 3e, 3f). These results indicated that dough improvers could protect gluten matrixes during frozen storage. Dough is a continuous matrix called the gluten network where the starch granules seemed to be scattered and some discontinuities were observed in the lax matrix surrounding the starch granules (Rojas et al., 2000). Berglund et al. (1991) found that the gluten matrix of dough after 24 weeks appeared less continuous, more disrupted, and separated from the starch granules leading the gluten strands to be also thinner. These results were similar to the work of Zounis (2002) found that starch obtained from dough improvers was not damaged after frozen at -20°C for 16 weeks.

CLSM images of frozen dough at two magnifications to allow a better interpretation of changes in dough structure (Figures 4a-4f). The fat phase (in red) is still visible after frozen and the protein and starch granule stains intensely grey. For frozen dough containing virgin coconut oil, the fat phase

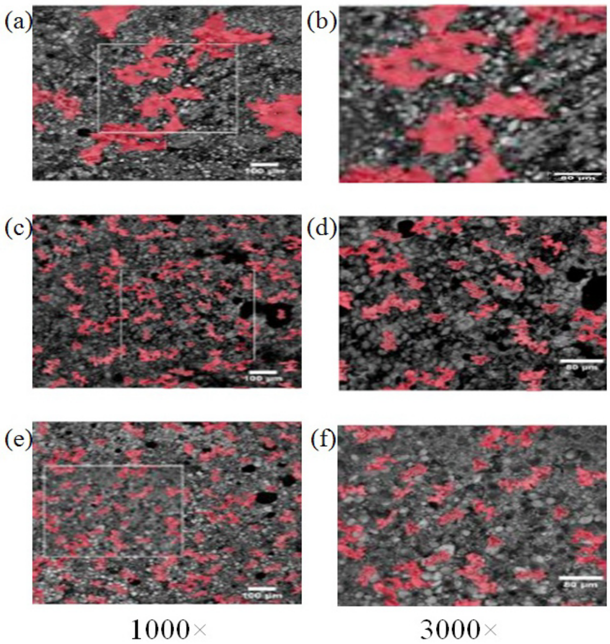

Figure 4. Confocal laser scanning microscope (CLSM) at 1000× and 3000× for 90 days; (a-b) virgin coconut oil, (c-d) shortening, and (e-f) 8% TR, 1.5% SE, 1.5% HPMC and virgin coconut oil

presented large area and was unevenly distributed (Figures 4a-4b). Microstructure of gluten was a rough, aggregated protein, similar to be not mixing enough. It should be note that gluten structure was damaged and starch granules were separated from gluten protein matrix. Depolymerization of glutenin aggregated during dough storage at −18°C and ice recrystallization caused to increase in gluten cross-linking (Ribotta *et al*., 2001; Ribotta *et al*., 2003b). Besides frozen dough containing shortening, the fat phase was evenly distributed, small area and higher smooth of gluten structure compared to frozen dough containing virgin coconut oil (Figures 4c-4d). It should be note that shortening was a good emulsifier. Li *et al*. (2004) reported that CLSM images of staining non polar fat were found in dough while staining polar fat also were found in both of dough and gas cell. Thus, polar fat was emulsifier property. The fat phase of frozen dough containing dough improver (8% TR, 1.5% SE, 1.5% HPMC) with virgin coconut oil was evenly distributed, small area and higher smooth of gluten structure compared to frozen dough containing virgin coconut oil (Figures 4e-4f). Starch granules of this dough formulation were embedded in gluten structure compared to frozen dough containing with shortening (Figures 4c-4d, 4e-4f). These results indicated that SE emulsifier evenly distributed the fat phase. HPMC also improved gluten matrix and smooth emulsion, thus, reduction of gluten cross-linking were caused by ice recrystallization. Sucrose esters can interact with starch and proteins to form complexes (Selomulyo and Zhou, 2007). Moreover, HPMC was a multiphase system like bread dough which it allows the dough to retain its uniformity and maintain the emulsion stability during bread making (Bell, 1990; Selomulyo and Zhou, 2007). Therefore, dough formulation as 8% TR, 1.5% SE, 1.5% HPMC and virgin coconut oil improved bread quality.

Conclusions

The effects of dough improvers (TR, SE, and HPMC) on the mixing stability, specific volume, textural and rheological properties of 1 and 90 days of frozen dough with virgin coconut oil and their bread quality were studied using response surface method. Addition of dough improvers (8% TR, 1.5% SE, and 1.5% HPMC) exhibited high bread quality. Its microstructure images exhibited evenly distributed fat phase and smaller and smoother gluten structure. All results suggested that, TR, SE, and HPMC were alternative dough improvers to improve frozen bread dough with virgin coconut oil.

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