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Comparison of Phagocytic Activity of Phagocytes in Whole Blood against Live and Heat-treated at 100°C *Saccharomyces cerevisiae*

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Abstract

Phagocytosis by neutrophils, eosinophils, and monocytes plays a critical role in inflammation through pathogen clearance and cytokine secretion. *Saccharomyces cerevisiae* is extensively used in food and beverage fermentation and typically undergoes heat treatment before consumption. While previous studies have examined phagocytic responses to yeast at baking temperatures (190°C), the effects of moderate heat treatment at 100°C commonly used in steaming and boiling remain poorly characterized. This study aimed to compare the phagocytic activity of neutrophils, eosinophils, and monocytes against live (RT) and heat-treated (ST; 100°C for 20 minutes) *S. cerevisiae* in human whole blood. Blood samples from 30 healthy volunteers (aged 19–24 years) with normal white blood cell counts were incubated with yeast suspensions (6.30×10^4 cells/μL) for up to 30 minutes. Viability assessment confirmed 96% viability for RT and 0% for ST. All phagocyte types engulfed both RT and ST yeast, indicating recognition of both live and heat-killed cells. At 30 minutes, the phagocytic percentages for neutrophils, eosinophils, and monocytes against RT were 88.76%, 74.11%, and 64.85%, respectively, compared to 83.77%, 53.57%, and 29.08% against ST. Notably, neutrophils against ST showed significantly higher phagocytic indices than against RT at 20–30 minutes, suggesting enhanced ingestion efficiency per cell despite reduced overall activation. Heat treatment significantly decreased phagocytic activity in eosinophils and monocytes. Neutrophils demonstrated superior phagocytic activity compared to eosinophils and monocytes against both RT and ST at all time points. These findings suggest that heat treatment at 100°C alters *S. cerevisiae* cell wall integrity, differentially affecting phagocyte responses and potentially reducing the immunogenic potential of heat-processed yeast products.

Keywords**:** *phagocytosis; Saccharomyces cerevisiae; neutrophil; eosinophil; monocyte; inflammation*

1. Introduction

Phagocytosis of *Saccharomyces cerevisiae* by phagocytes (neutrophil, monocyte and eosinophil) is a fundamental protective process in the immune response (Meesilp & Suksoem, 2025). The dysregulated or persistent phagocytic response can contribute to chronic inflammation and disease (Herrero-Cervera et al., 2022). If neutrophils remain activated at a site of chronic inflammation, they can release excessive enzymes and form neutrophil extracellular traps (NETs), which may damage host tissues, contributing to the pathology of diseases like atherosclerosis and chronic inflammatory disease. The activation of monocytes results in the release of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), and the presentation of antigens to T lymphocytes (T cells) to initiate an adaptive immune response (Phuengmaung

et al., 2024). Although eosinophils play a major role in parasitic infections, they can cause inflammation through eosinophil-derived cytotoxic mediators (Lombardi et al., 2022). The phagocytes recognize *Saccharomyces cerevisiae* through their pattern recognition receptors (PRRs). These receptors bind to pathogen-associated molecular patterns (PAMPs), such as β-glucans, mannans, and chitin on the yeast cell wall. Although both live and killed yeast possess these PAMPs, the physical state of the cell particularly following heat treatment can alter their accessibility. *Saccharomyces cerevisiae* was cultured on yeast malt medium to support optimal growth and consistent expression of cell wall components (Rungsardthong et al., 2023), as nutrient conditions can influence the distribution and exposure of PAMPs. Such standardization is essential because phagocytic responses depend heavily on the extent to which PAMPs are available for recognition by pattern recognition receptors (PRRs) (Chen et al., 2025). Accordingly, phagocytic activity toward *S. cerevisiae* can differ between live and heat-treated yeast due to changes in PAMP accessibility. In live yeast cells, many PAMPs are partially shielded by an outer layer of mannoproteins, reducing their immediate detection by the immune system. By disrupting this outer layer, heat treatment at 100ºC for 5 to 10 minutes makes the β-glucans more accessible to the immune system's Dectin-1 receptors (Aiamsa-ard et al., 2025). Studies comparing the phagocytosis of live and killed *Saccharomyces cerevisiae* have shown inconsistent results, which often depend on the specific strain of yeast, the type of phagocyte, and the killing method. Intracellular killing of live *Saccharomyces cerevisiae* was consistently and significantly more rapid by monocytes than by neutrophils (Schuit, 1979). *Saccharomyces cerevisiae* with heat-treated at baking temperature (190ºC) effects reduction of the phagocytic activity of neutrophil compared with live yeast cells (Chobchuenchom, 2024). However, heat treatment is essential for inactivating the yeasts in most food and beverage products that use *Saccharomyces cerevisiae*. Heat treatment at 100ºC for 20 minutes by streaming or boiling is a common method used for inactivating yeast such as steamed buns. Therefore, the effect of *Saccharomyces cerevisiae* heat-treated at 100°C on phagocytic activity should be clarified by focusing on yeast cell wall integrity and recognition alteration.

Although previous studies have examined phagocytosis of *Saccharomyces cerevisiae* heat-treated at baking temperature (190°C), focusing primarily on neutrophil responses, a significant gap exists in understanding how moderate heat treatment at 100°C commonly used in boiling and steaming processes affects the phagocytic activity of different phagocyte types. Given that eosinophils and monocytes also contribute to chronic inflammation through distinct mechanisms, and that heat treatment at different temperatures may variably alter yeast cell wall integrity and PAMP exposure, comprehensive characterization of multi-phagocyte responses to 100°C treated yeast is essential for understanding the immunogenic potential of widely consumed heat-processed yeast products.

2. Objectives

This study aimed to compare the phagocytic activity of neutrophils, eosinophils, and monocytes against live and heat-treated at 100°C *Saccharomyces cerevisiae* in human whole blood.

3. Materials and Methods

3.1 Participants and Sample Collection

The sample size (n = 8) was calculated using a power of 0.80, α at 0.05 and effect size d at 1.36 according to the data from previous study (Chobchuenchom, 2024) by using G*Power software version 3.1.9.4. However, this study was conducted on healthy male (n = 8) and female (n = 22) volunteers who were aged between 19-24 years. After signing the informed consent form, 4 mL of intravenous blood was collected into heparinized BD vacutainer tubes (BD Biosciences, Franklin Lakes, NJ, USA) and transported to the laboratory immediately. Then, the white blood cell count was performed by diluting blood 1:20 with 3% (v/v) acetic acid in distilled water or Turk reagent by using white cell pipette, counting under microscope using hemacytometer and calculating the WBC count in 10^9 cells/L. For determination of WBC differential, a drop of blood was thinly spread over a glass slide, air dried, and stained with the Wright-Giemsa technique. All participants who showed normal white blood cell count and differential percentage were further calculated for the absolute number of neutrophils, eosinophils and monocytes from the percentage of each white blood cell and the WBC count.

3.2 Preparation of Live (RT) and Heat-Treated (100°C; ST) *Saccharomyces cerevisiae* Suspensions

The industrial strain of *Saccharomyces cerevisiae* used in this study was kindly provided by the Department of Clinical Microbiology, Faculty of Medical Technology, Rangsit University. The frozen stock, preserved in 25% (v/v) glycerol, was revived and streaked onto potato dextrose agar (PDA) plates to obtain a single colony (Puangwerakul et al., 2025). This colony was subsequently subcultured onto fresh PDA and incubated at 30°C for 48 hours. After incubation, the yeast cells were harvested and washed twice with phosphate buffer (pH 7.2) by centrifugation. The final suspension was adjusted to 6.3×10^4 cells/µL and designated as the room-temperature (RT; live) yeast preparation (Guidry & Trelles, 1962).

To generate the heat-treated (ST) preparation, 2 mL of the RT suspension was placed in a hot-air oven and heated at 100°C for 20 minutes under dry conditions, following previously described inactivation procedures (Chobchuenchom, 2024).

3.3 Viability Study

The viability of *Saccharomyces cerevisiae* RT and ST was determined by using the traditional methylene blue method (Matsumoto et al., 2022). Briefly, a volume of 100 µL of each RT and ST yeast suspension was mixed with 100 µL of methylene blue (0.1 g/dL) in distilled water, left for 1 minute, dropped into the counting chamber and covered with a cover slip. Then, the dead yeast cells (blue stained) and viable yeast cells (non-blue stained) were counted under a 400X light microscope in triplicate. Finally, the viability percentage (%) was calculated by comparing the viable yeast cell count to the total number of 100 viable and non-viable yeast cells.

3.4 Phagocytic Activity Study

The activity of neutrophils, eosinophils, and monocytes was assessed in whole blood by modifying the method described previously (Minarova et al., 2021, Chobchuenchom, 2024). Briefly, one mL of heparinized whole blood was obtained from volunteers (n = 30) and 100 µL of yeast suspension of live *Saccharomyces cerevisiae* prepared at room temperature (RT) or heat-treated *Saccharomyces cerevisiae* at 100ºC (ST) and incubated at 37ºC in a water bath with shaking condition. At incubation time of 0, 5, 10, 15, 20, 25 and 30 minutes, blood samples were taken and prepared as thin blood smear on a glass slide using the conventional method and allowed the smear to air dry completely. After that, the slide was fixed with absolute methanol for at least 30 seconds, flooded and stained the slide with the Wrights-Giemsa stain dye for 4 minutes, flooded and covered the slide with phosphate buffer (pH 6.8) for 4 minutes. Finally, rinsed slide with running tap water. Then, differential count of each phagocyte with and without phagocytosis as well as the number of ingested yeasts from a total number of 50 phagocytes was counted in triplicate. The phagocytic percentage and phagocytic index that represented the average numbers of the ingested yeast per phagocyte were calculated by using the following calculation equations, respectively.

Phagocytic percentage (%) = numbers of each phagocyte with phagocytosis x 100/total numbers of each phagocyte

Phagocytic index = total numbers of ingested yeast by each phagocyte/ total numbers of each phagocyte

For example, if 40 neutrophils have 120 engulfed particles from a total number of 50 neutrophils, the phagocytic percentage and index will be 40 x 100/50 = 80 % and 120/50 = 2.4, respectively.

3.5 Statistic Analysis

Descriptive statistics were calculated for mean, standard deviation, standard error of the mean and 95% confidence intervals of the means of phagocytic percentage and phagocytic index. The normal distribution of the data was determined by the Shapiro-Wilk test. The Wilcoxon signed-rank test for matched pair difference (for non-parametric data) or paired t-test (for parametric data) was used for comparing phagocytic percentage and phagocytic index between RT and ST. All data were analyzed by using IBM SPSS (An IBM Company, USA).

3.6 Ethics

This study was approved by the Ethics Review Board for Human Research of Rangsit University, Thailand (Certificate of approval number: RSUERB2024-038 on March 4, 2024).

4. Results

4.1 White Blood Cell Count and Absolute Count

As illustrated in Table 1, the means of total white blood cell count, absolute neutrophil count, absolute

lymphocyte count, absolute eosinophil count, absolute basophil count and absolute monocyte count were within normal values that were 5.78, 2.99, 2.33, 0.16, 0.01 and 0.27 x 10^9 cells/L, respectively.

4.2 Viable Yeast Cell Count and Viability Percentage of *Saccharomyces cerevisiae* RT and ST

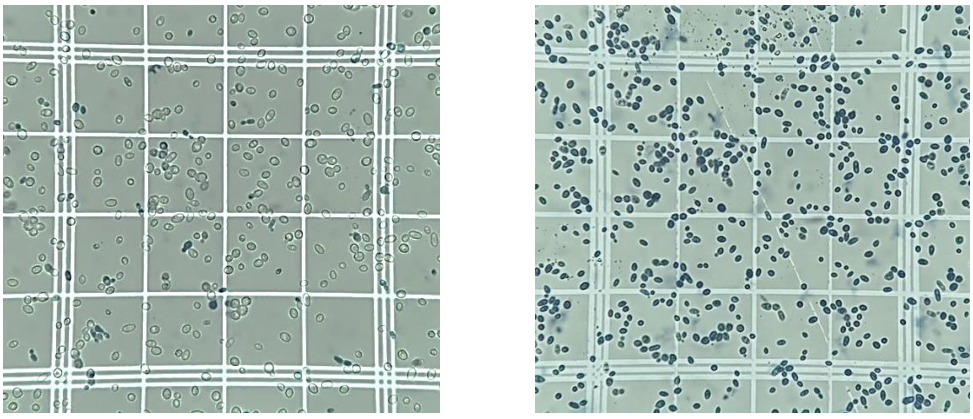
The cell suspension of *Saccharomyces cerevisiae* with 6.3 x 10^4 cells/µL total cell count was used for dead cells or cells with compromised membranes. preparation of RT and ST. As shown in Table 2, by using traditional methylene blue staining, viable yeast cell count of *Saccharomyces cerevisiae* RT was 5.73 x10^4 cell/ µL with percentage of viable yeast cells of 96% (Figure 1a). On the other hand, there was no viable yeast cell found in *Saccharomyces cerevisiae* ST suspension (Figure 1b). All ST yeast cells were stained with methylene blue which indicated that they were

Table 1 White blood cell count and absolute count of 30 healthy volunteers

	Range [x10^9 cell/L]	Mean [x10^9 cell/L]	Standard Deviation [x10^9 cell/L]	Normal value [x10^9 cell/L]	References
White blood cell count	4.50–8.80	5.78	1.21	4.5–11	Cheng et al., 2004
Absolute neutrophil count	1.51–4.97	2.99	0.79	2.0–8.0	Pagana et al., 2019
Absolute lymphocyte count	1.46–3.56	2.33	0.49	1.0–4.0	
Absolute eosinophil count	0–0.47	0.16	0.10	0.05–0.5	
Absolute basophil count	0–0.015	0.01	0.03	0.025–0.1	
Absolute monocyte count	0.11–0.50	0.27	0.12	0.1–0.7	

Table 2 Viable yeast cell count and viability percentage of RT and ST

Saccharomyces cerevisiae	Viable yeast cell count (cell/µL)	Viability percentage (%)
RT	5.73 x10^4	96
ST	0	0



(a) (b)

Figure 1 Methylene blue viability staining of *Saccharomyces cerevisiae* RT (a) and ST (b)

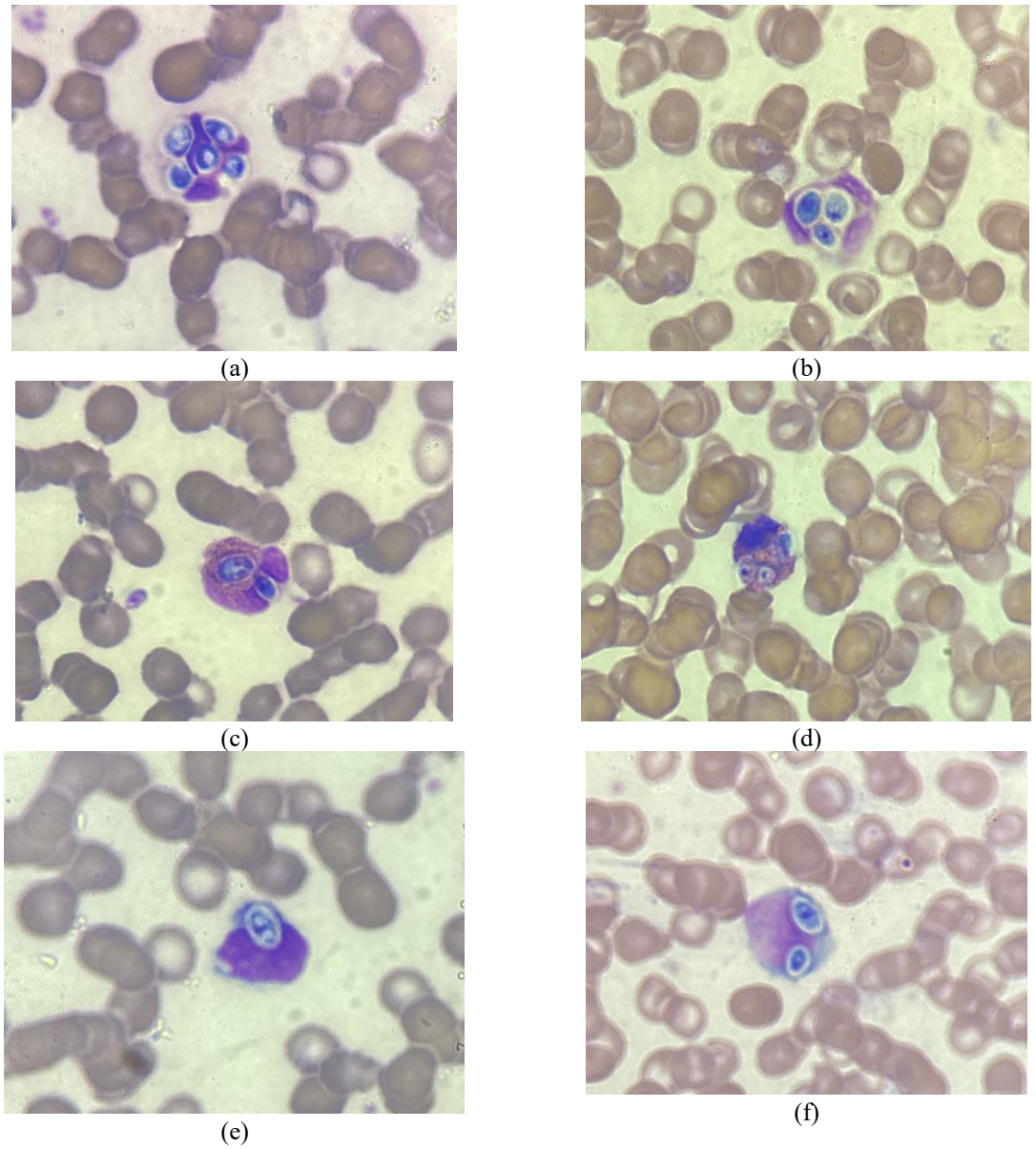


Figure 2 Phagocytosis of Saccharomyces cerevisiae at 25 minutes of incubation showing differential cell wall recognition: (a, b) neutrophils engulfing RT and ST yeast; (c, d) eosinophils recognizing RT and ST; (e, f) monocytes phagocytosing RT and ST

4.3 Phagocytosis of Neutrophils, Eosinophils and Monocytes Against *Saccharomyces cerevisiae* RT and ST

The experiments were performed to compare the phagocytic activities of neutrophils, eosinophils and monocytes in whole blood against two types of *Saccharomyces cerevisiae*, namely RT and ST suspension with 96% and 0 % viability, respectively. All types of phagocytes in whole blood could phagocytose both RT and ST, showing that live yeasts, dead yeasts, or yeasts with compromised membranes can be recognized by phagocytes. Examples of the phagocytes showing phagocytosis against RT and ST are shown in Figure 2.

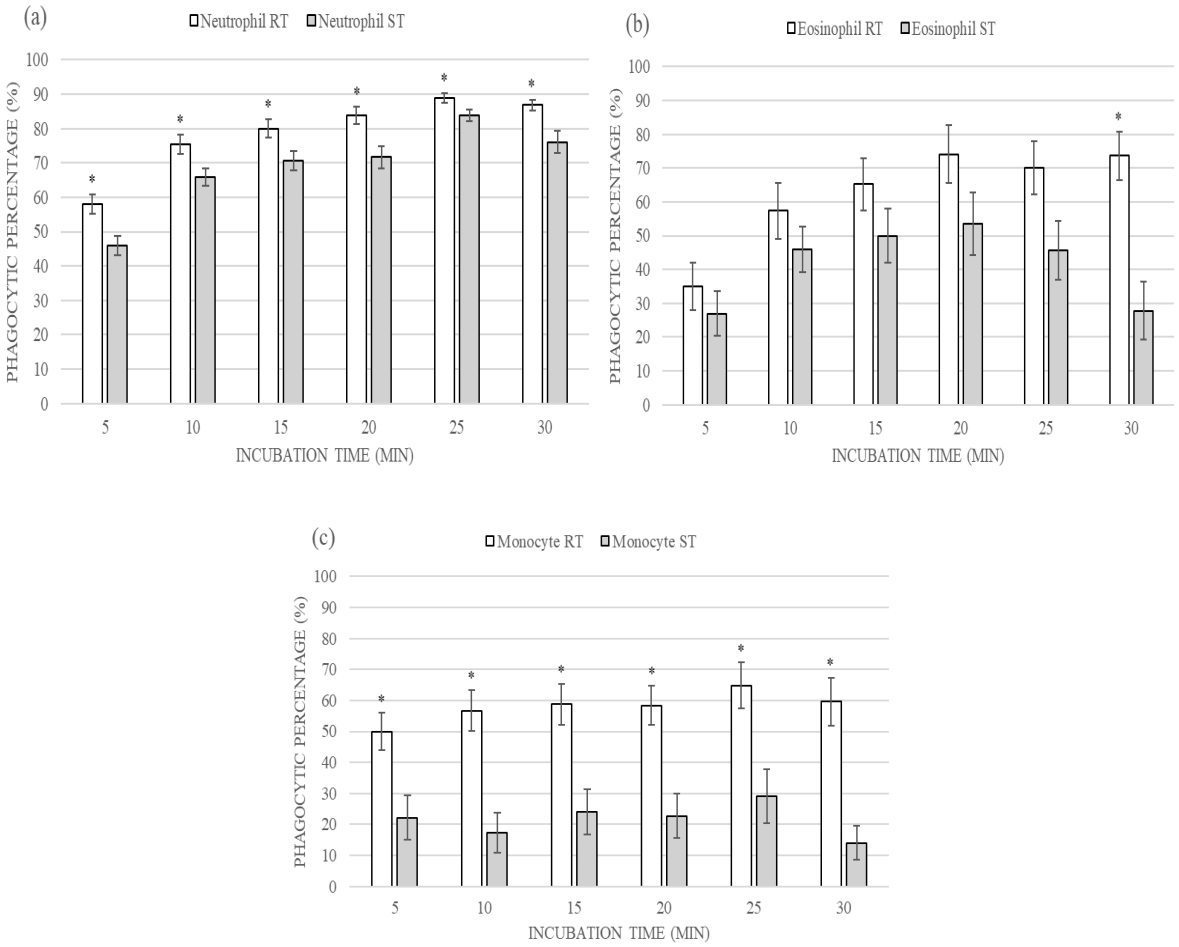


Figure 3 Mean and standard error of the mean (SEM) of phagocytic percentage of neutrophils (a), eosinophils (b) and monocytes (c) against Saccharomyces cerevisiae RT and ST at 0, 5, 10, 15, 20, 25, and 30 minutes of incubation (n = 30)

4.4 Comparison of Phagocytic Percentage and Index of Neutrophils, Eosinophils and Monocytes Against *Saccharomyces cerevisiae* between RT and ST

As illustrated in Table 3 and Figure 3, the highest phagocytic percentage of neutrophils, eosinophils and monocytes against RT were 88.76%, 74.11% and 64.85% and against ST were 83.77%, 53.57% and 29.08%, respectively. Both neutrophil and monocytes showed the highest phagocytic percentage against RT and ST at 25 minutes of incubation, whereas eosinophil showed the highest percentage at 20 minutes of incubation. At 5, 10, 15, 20, 25 and 30 minutes of incubation, both neutrophils and monocytes showed significantly higher phagocytic percentage against RT than ST ($p < 0.05$) with medium effect size (Cohen's d, .202 to .679) for neutrophil and medium to large effect size (.590 to .990) for monocyte. Although at 30 minutes of incubation, the phagocytic percentage of eosinophil showed a significant difference between against RT and ST (Cohen's d = .843), no significant difference was found at other incubation times with small effect size (Cohen's d,.186 to .395).

Table 3 Comparison of phagocytic percentage of phagocytes against *Saccharomyces cerevisiae* RT and ST at various times of incubation

Phagocytes	Incubation time [minutes]	Phagocytic percentage				Cohen's d	*p*-value
		RT		ST			
		mean±SD	95%CI	mean±SD	95%CI		
Neutrophil	5	57.96 ± 15.70	52.01–63.81	45.95 ± 15.21	40.27–51.63	.679	.001*
	10	75.52 ± 15.41	69.76–81.27	65.81 ± 13.54	60.75–70.87	.538	.006*
	15	79.97 ± 14.32	74.63–85.32	70.59 ± 15.74	64.71–76.47	.439	.023*
	20	83.89 ± 14.09	78.63–89.15	71.61 ± 18.00	64.89–78.33	.570	.004*
	25	88.76 ± 07.44	85.98–91.54	83.77 ± 9.35	80.28–87.26	.387	.043*
	30	86.79 ± 12.94	81.96–91.63	76.07 ± 17.61	69.50–82.65	.484	.013*
Eosinophil	5	34.97 ± 34.13	20.55–49.38	26.94 ± 32.00	13.43–40.46	.191	.360
	10	57.41 ± 37.32	42.64–72.17	45.83 ± 38.34	30.67–61.00	.186	.343
	15	65.16 ± 34.62	48.95–81.36	50.00 ± 35.46	33.40–66.59	.325	.162
	20	74.11 ± 31.95	55.66–92.55	53.57 ± 34.70	33.54–73.61	.395	.163
	25	70.00 ± 37.31	53.87–86.13	45.65 ± 41.81	27.57–63.73	.380	.119
	30	73.58 ± 31.63	58.78–88.39	27.83 ± 38.43	09.85–45.82	.843	.006*
Monocyte	5	49.94 ± 29.27	37.29–62.60	22.17 ± 34.31	7.34–37.01	.632	.006*
	10	56.69 ± 35.28	43.00–70.37	17.26 ± 33.79	04.16–30.63	.757	.001*
	15	58.74 ± 34.12	45.29–72.28	24.07 ± 38.21	08.96–39.19	.684	.002*
	20	58.31 ± 32.26	45.28–71.34	22.76 ± 36.02	08.21–37.31	.799	.001*
	25	64.85 ± 30.68	49.08–80.63	29.08 ± 35.44	10.86–47.31	.590	.010*
	30	59.57 ± 38.83	43.54–75.60	14.00 ± 27.50	02.65–25.35	.990	.001*

Note: * Significant difference ($p < 0.05$)

Table 4 Comparison of phagocytic index of phagocytes against *Saccharomyces cerevisiae* RT and ST at various times of incubation

Phagocytes	Incubation time [minutes]	Phagocytic index				Cohen's d	*p*-value
		RT		ST			
		mean±SD	95%CI	mean±SD	95%CI		
Neutrophil	5	1.09 ± 0.57	0.87–1.30	0.93 ± 0.38	0.79–1.07	.257	.169
	10	1.85 ± 1.11	1.44–2.26	1.60 ± 0.45	1.43–1.77	.265	.634
	15	2.25 ± 1.28	1.77–2.72	2.10 ± 0.69	1.84–2.36	.115	.880
	20	2.44 ± 1.38	1.93–2.96	2.61 ± 1.14	2.19–3.03	–.102	.339
	25	2.40 ± 0.83	2.09–2.71	2.65 ± 0.76	2.37–2.94	–.413	.021*
	30	2.56 ± 1.38	2.04–3.08	2.97 ± 1.35	2.46–3.47	–.252	.036*
Eosinophil	5	0.62 ± 0.78	0.29–0.95	0.72 ± 0.79	0.29–1.14	–.079	.704
	10	0.97 ± 0.81	0.60–1.34	0.57 ± 0.56	0.31–0.83	.331	.055
	15	1.80 ± 1.04	1.19–2.41	1.13 ± 1.03	0.54–1.73	.726	.073
	20	1.32 ± 0.96	0.76–1.88	1.07 ± 0.87	0.57–1.57	.198	.473
	25	1.49 ± 1.36	0.90–2.08	0.89 ± 0.94	0.48–1.30	.356	.102
	30	1.71 ± 1.12	1.19–2.24	0.51 ± 0.74	0.16–0.86	.877	.001*
Monocyte	5	0.89 ± 0.67	0.60–1.18	0.83 ± 1.55	0.17–1.50	.034	.372
	10	1.32 ± 1.19	0.86–1.78	0.90 ± 1.33	0.29–1.34	.179	.053
	15	1.57 ± 1.18	1.10–2.03	0.82 ± 2.10	0.32–1.98	.447	.028*
	20	1.53 ± 1.22	1.03–2.03	0.71 ± 1.13	0.25–1.18	.713	.002*
	25	1.59 ± 1.56	0.69–2.49	0.28 ± 0.58	0.06–0.62	.534	.021*
	30	1.81 ± 1.42	1.24–2.37	1.04 ± 1.65	0.38–1.69	.402	.047*

Note: * Significant difference ($p < 0.05$)

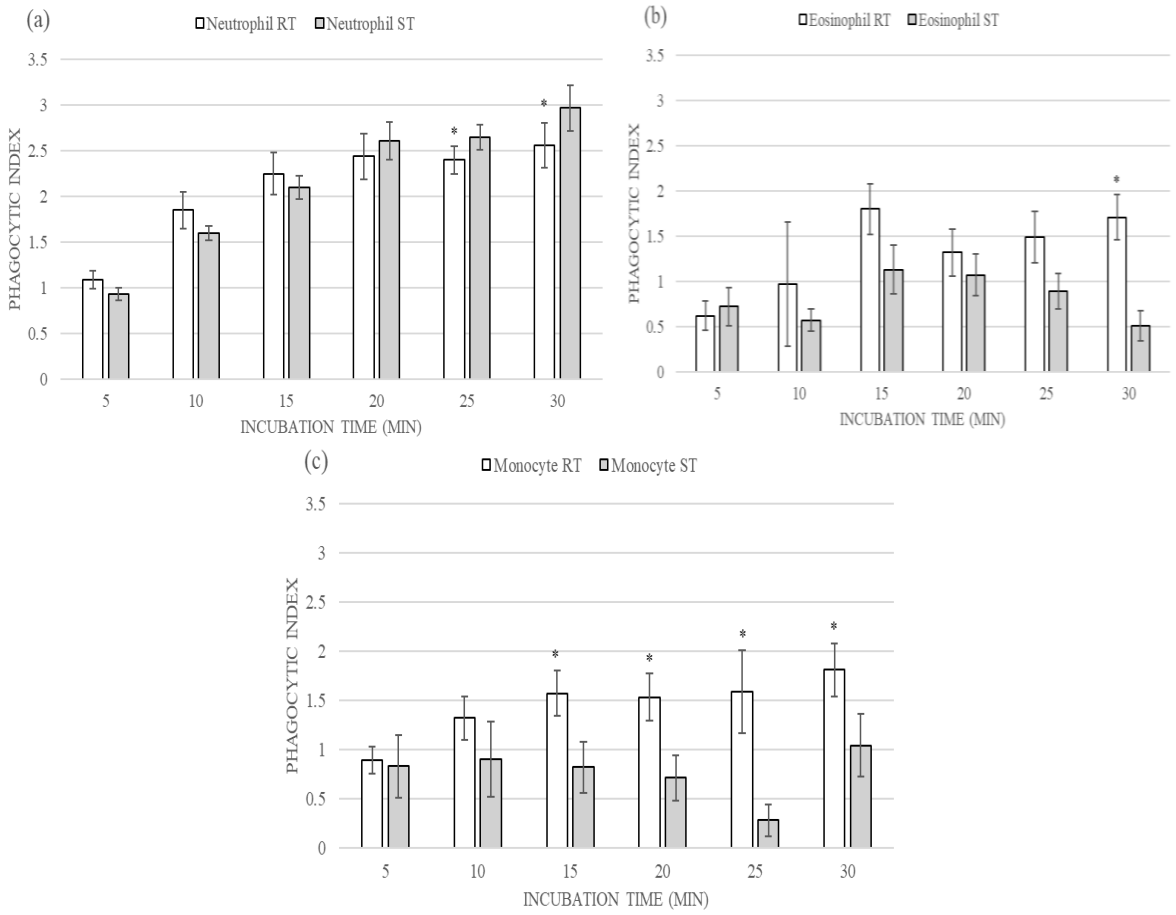


Figure 4 Mean and standard error of the mean (SEM) of phagocytic index of neutrophils (a), eosinophils (b) and monocytes (c) against Saccharomyces cerevisiae RT and ST at 0, 5, 10, 15, 20, 25, and 30 minutes of incubation (n = 30).
* Significant difference ($p < 0.05$)

The highest phagocytic indices of neutrophils, eosinophils, and monocytes against RT were 2.56, 1.80, and 1.81, and those against ST were 2.97, 1.13, and 1.04, respectively. Both neutrophil and monocytes showed the highest phagocytic index against RT and ST at 30 minutes of incubation, whereas eosinophils showed the highest percentage at 15 minutes of incubation (Table 4 and Figure 4). In contrast to the reduction of phagocytic percentage, at 25 and 30 minutes of incubation, the phagocytic index of neutrophil against ST was significantly higher than RT (Cohen's d = –.413 and –.252, respectively). At 30 minutes of incubation, the phagocytic index of eosinophils against RT was significantly higher than against ST, a difference characterized by a high effect size (Cohen's d = 0.877). At 15, 20, 25 and 30 minutes of incubation, the phagocytic index of monocytes against RT was significantly higher than ST (Cohen's d = .447, .713, .534 and .402, respectively). No significant differences in phagocytic index between RT and ST were observed in any other experiments ($p > 0.05$) with small to medium effect size.

4.5 Comparison of Phagocytic Percentage and Index among Neutrophils, Eosinophils and Monocytes

Comparisons were made to assess phagocytic percentage and index among neutrophils, eosinophils and monocytes at various times of incubation. The phagocytic percentage and index against RT and ST of neutrophils were significantly higher than eosinophils and monocytes at all incubation times ($p < 0.05$) with Cohen's d values ranging between .401 to 1.922. On the other hand, there was no significant difference when compared phagocytic percentage and index against RT and ST between eosinophils and monocytes at any incubation times ($p > 0.05$) with Cohen's d values ranged between .002 to .846 (Table 5 and Table 6).

Table 5 Comparison of phagocytic percentage among neutrophils, eosinophils and monocytes against *Saccharomyces cerevisiae* RT and ST at various times of incubation

Phagocytes comparison	***Saccharomyces cerevisiae***		**Incubation time [minutes]**					
			5	**10**	**15**	**20**	**25**	**30**
Neutrophils and eosinophils	RT	*p*-value	.001*	.009*	.014*	.007*	.012*	.008*
		Cohen's d	.725	.540	.570	.602	.522	538
	ST	*p*-value	.002*	.001*	.010*	.028*	.001*	.001*
		Cohen's d	.683	.765	.550	.448	.980	.934
Neutrophils and monocytes	RT	*p*-value	.041*	.012*	.006*	.007*	.001*	.002*
		Cohen's d	.414	.512	.560	.562	.747	.645
	ST	*p*-value	.002*	.001*	.001*	.001*	.001*	.001*
		Cohen's d	.672	1.609	1.312	1.176	1.864	1.825
Eosinophils and monocytes	RT	*p*-value	.099	.992	.207	.098	.946	.497
		Cohen's d	–.343	.002	.292	.426	–.013	.135
	ST	*p*-value	.496	.156	.124	.080	.241	.113
		Cohen's d	–.148	.266	.294	.344	.295	.361

Note: * Significant difference ($p < 0.05$)

Table 6 Comparison of phagocytic index among neutrophils, eosinophils and monocytes against *Saccharomyces cerevisiae* RT and ST at various times of incubation

Phagocytes comparison	***Saccharomyces cerevisiae***		**Incubation time [minutes]**					
			5	**10**	**15**	**20**	**25**	**30**
Neutrophils and eosinophils	RT	*p*-value	.001*	.001*	.040*	.003*	.003*	.014*
		Cohen's d	.722	.689	.467	.777	.633	.499
	ST	*p*-value	.036*	.001*	.001*	.002*	.001*	.001*
		Cohen's d	.401	1.113	1.172	.748	1.396	1.131
Neutrophils and monocytes	RT	*p*-value	.014*	.023*	.001*	.001*	.001*	.003*
		Cohen's d	.507	.455	.724	.765	.742	.616
	ST	*p*-value	.027*	.031*	.017*	.001*	.001*	.001*
		Cohen's d	.442	.415	.473	1.483	1.922	1.212
Eosinophils and monocytes	RT	*p*-value	.076	.591	.089	.922	.411	.860
		Cohen's d	–.371	.109	.401	.024	–.219	.035
	ST	*p*-value	.103	.693	.266	.100	.846	.852
		Cohen's d	–.363	.083	–.228	.377	.048	–.041

Note: * Significant difference ($p < 0.05$)

5. Discussion

Phagocytosis relies on the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) on phagocytic cells, triggering engulfment and destruction of pathogens as well as leading to the production of inflammatory cytokines. The cell wall of *Saccharomyces cerevisiae* is composed mainly of polysaccharides and proteins, which act as PAMPs, namely β-glucans and α-mannans (Remondo et al., 2009). The β-glucans are glucose polymers that form the inner, structural backbone of the cell wall. They are the most potent PAMPs in yeast and are typically found as β-1,3-glucan (the main component) and β-1,6-glucan. In a live yeast cell, these β-glucans are largely shielded from the host immune system by an outer layer. Mannoproteins (α-mannans) are heavily glycosylated proteins that form the outermost layer of the cell wall (Aguilar-Uscanga & François, 2003). They are rich in mannose residues, which are also recognized by host immune cells (Javmen et al., 2017). Phagocytes contain a variety of PRRs that specialize in recognizing fungal PAMPs. The most important of these receptors are as follows: Dectin-1, a C-type lectin receptor is the primary PRR for β-glucans. When Dectin-1 binds to β-glucans, it triggers a signaling cascade that leads to phagocytosis, the production of pro-inflammatory cytokines (e.g., TNF-α, IL-6), and the generation of reactive oxygen species (ROS) to kill the invading microbe. TLR2 and TLR4 are Toll-like receptors that are involved in fungal recognition. They can recognize components of the yeast cell wall, often working synergistically with Dectin-1 to amplify the inflammatory response. Mannose Receptor (CD206), a C-type lectin receptor that recognizes the terminal mannose residues on the α-mannans of the yeast cell wall (Andriana et al., 2025). The differences in α-mannan and β-1,3-glucans chain lengths affected their capacity to be recognized by PRRs as well as affected the downstream signaling and mobilization of effector T-helper cell subsets (Rawling et al., 2023). Disrupting the outer mannoprotein layer by heat treatment, particularly at around 100°C for 5 to 10 minutes, alters the physical structure of the yeast cell wall and makes the β-glucans more accessible to the Dectin-1 receptor, leading to a more potent and efficient phagocytic response (Cheng et al., 2024). In this study, the live *Saccharomyces cerevisiae* suspension (RT) was subjected to heat treatment at 100°C for 20 minutes to generate the heat-treated yeast preparation (ST). This heating duration was selected because it reflects standard yeast inactivation conditions commonly used in steamed bun production (Suebwongsa et al., 2024). As shown in Figure 1, all heat-treated yeast cells stained with methylene blue, indicating loss of membrane integrity and confirming that the ST preparation was fully non-viable. Notably, the methylene blue staining reflects membrane permeabilization rather than cell wall destruction.

The phagocytic percentage of neutrophils was consistently and significantly higher against RT compared to ST yeast at all incubation times with Cohen's d values ranged from small to medium effect size. However, there was no significant difference in the phagocytic index between RT and ST yeast at initial response (5–20 minutes). Cohen's d values were very small (less than 0.3). On the other hand, the phagocytic index of neutrophils showed a slight but significant increase against ST at later incubation times (25 and 30 minutes). The negative Cohen's d values (–0.413 and –0.252, respectively) reflect this increase in the ST, with a small to medium effect size. These results suggest that heat treatment at 100°C enhanced the phagocytic efficiency of neutrophils (indicated by an increase in the number of ingested yeasts per cell), despite a small reduction in the overall percentage of phagocytosing cells. Heat treatment at 100°C is likely denatures or alters the outer α-mannans and unmasks β-glucans, resulting in a higher phagocytic index. In contrast, this structural modification reduces the initial activation signal, leading to a lower overall percentage of neutrophils becoming activated and initiating phagocytosis (Cheng et al., 2024). Although no statistically significant differences in the phagocytic percentage or phagocytic index of eosinophils were observed between the RT and ST groups during the first 5 to 25 minutes of incubation, a significant difference emerged at 30 minutes. At this time point, both the phagocytic percentage and phagocytic index for RT were significantly higher than those for ST, with a large effect size (high Cohen's d).

These findings indicate that heat treatment at 100°C markedly reduces eosinophil phagocytic activity (Samuthpongtorn et al., 2025). The phagocytic percentage of monocytes was consistently and significantly higher against RT compared to ST at all incubation times, with a particularly large effect. Moreover, the phagocytic index of monocytes against RT yeast was consistently

and significantly higher than against ST yeast at most incubation times. These findings suggest a consistent reduction in the phagocytic efficiency of monocytes against heat-treated yeast.

The data in Tables 5 and 6 show that neutrophils are consistently more effective at phagocytosis than eosinophils for both RT and ST with range from medium to large effect size, confirming that the difference in phagocytic activity is substantial. Similar to the comparison with eosinophils, neutrophils are also consistently more effective at phagocytosis than monocytes for both RT and ST. All *p*-values are statistically significant. Cohen's d values for RT yeast are consistently in the medium to large range, indicating a clear, meaningful difference. These results differ from a previous report which suggested that human monocyte ingested *Saccharomyces cerevisiae* more rapidly than neutrophils (Schuit, 1979). This may result from differences in phagocyte preparation and condition for phagocytic activity testing. For ST, the Cohen's d values jump to a very large effect size at the 20, 25, and 30 minutes, with values of 1.483, 1.922, and 1.212, respectively. These findings suggest that heat treatment at 100°C reduces the phagocytic efficiency of monocytes. In contrast to the other comparisons, there is no statistically significant difference in the phagocytic percentage and index between eosinophils and monocytes for either RT or ST. All *p*-values are greater than 0.05. The Cohen's d values are all very low (mostly less than 0.2), indicating a small and likely practically insignificant difference. This suggests that phagocytic activities of eosinophils and monocytes are comparable.

It is worth noting that the phagocytic percentage and index of eosinophils and monocytes against RT and ST show high SD values. This indicates high variability and less consistency of the data. This suggests that the response of eosinophils and monocytes to *Saccharomyces cerevisiae* is not uniform across all individuals. This could be due to individual differences in opsonin levels in the tested whole blood, or the limitations of reading the phagocytic activity on blood smear. The process of manually counting cells and internalized particles under a microscope is highly susceptible to human error and subjectivity and may result in counting and interpretation biases affecting the reliability of the results (Gupta-Wright et al., 2017). However, the data from this study suggests that the temperature and duration of heat treatment may alter yeast cell wall integrity, leading to subsequent changes in phagocytic activity (Sripakdee et al., 2024). Therefore, further research is needed to investigate how varying temperatures and durations of yeast inactivation in food production affect cell wall integrity and subsequent phagocytic activity.

6. Conclusions

Heat treatment at 100ºC affects *Saccharomyces cerevisiae* cell wall integrity and subsequent phagocytic activity. Heat treatment has a dual effect on neutrophils. While it reduces phagocytic percentage, it significantly enhances the number of yeast particles each activated neutrophil can ingest. The phagocytic activity (both percentage and index) of monocytes and eosinophils was significantly reduced against heat-treated yeast. Monocytes and eosinophils have comparable phagocytic activity toward both live and heat-treated yeast.

7. Abbreviations

Abbreviation	Full Term
CI	confidence interval
cells/L	cells per liter
IL-1β	interleukin-1 beta
IL-6	interleukin-6
mL	milliliter
NETs	neutrophil extracellular traps
PAMPs	pathogen-associated molecular patterns
PDA	potato dextrose agar
PRRs	pattern recognition receptors
ROS	reactive oxygen species
RT	room temperature
SD	standard deviation
SEM	standard error of the mean
ST	heat-treated at 100°C
TNF-α	tumor necrosis factor alpha
v/v	volume by volume
WBC	white blood cell

8. CRediT Statement

Wimol Chobchuenchom: Conceptualization, methodology, resources, laboratory experiments, statistical analysis, data curation, visualization, writing – review & editing.

9. Reference

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