Anti-mycotoxigenic properties of “Fino” using the modified zinc-yeast

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ABSTRACT

A traditionally prepared Saccharomyces yeast helps to improve nutritive food qualities and health impacts. Zinc-Yeast was formulated with the aim to enhance bakery products, extend shelf-life, improve safety, and diet-support by an essential mineral (Zn). A strain NRC112 was pre-treated to evaluate Zn-support optimum conditions. The best uptake was recorded at 1623 µg/g using zinc-sulphate. For safety-production increment; Zn-yeast effect on toxigenic fungi and aflatoxins was estimated on liquid media and diffusion tests. Fino was designed for comparing the differences between Zn-yeast and the control. In comparing the result of the paste and bread; Zn-yeast was more effective in aflatoxins reduction than the control. Organoleptic and chemical properties of Fino was also investigated to explore if Zn-yeast application distinct compared to the control. The Fino manufacturing by Zn-yeast was not significantly different from control. The sensory evaluation results were so close in the two types. Zn-yeast confers Fino fungal resisting properties and long shelf-life.

1. Introduction

Saccharomyces has a protracted story in the food application. It is a classical yeast strain in the bread manufacturing to give the quality and organoleptic properties of the product. As a result of chemical reactions that occur due to yeast present in the paste, produced gases award the required characteristics of aeration and porosity. Also in these reactions, some compound flavours could results gives an amazing taste and a sweet flavor to the bread (Sillerová et al., 2012). The yeast becomes a nutritional substance provide a complete protein, B vitamins, and Iron. It could be applied in coated bio-film for bakery products (Shehata, Badr, Abdel-Razek, Hassanein, & Amra, 2017).

Zinc is a fundamental element represented as traces in food material and living organism cells. Zinc importance for human & animals tissues, also for nutrition & public health, was first shown by Gadd and Mowll (1983). It has a significant function in nucleic acid synthesis as well as cell replication. Furthermore, zinc could be a critical function in enzyme activity. The lack of zinc from biological membranes causes function loss. Milder deficiency in zinc give a share in the physical and neuro-psychological damages progress and growing the life-threatening infections tendency in young children (Lyons et al., 2000). Trace elements supplement offered in two forms, inorganic salts or organic-metal substrates. Inorganic salts are not a ready-form for biological activities as the organic form. Likewise, retention of an organically bound mineral occurs higher than for inorganic salts. Yeast fortification by zinc offers an organic-zinc-form which is more suitable for absorption in the biological systems. Therefore, zinc must support the nutritional intake of living organisms, in this respect, the presence of yeast fortified with zinc not only offer the preferable

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characteristics of the bakery products, but it could be functioned as a functional food.

Toxicogenic fungi are the most food hazard during production, transportation, and storing time (Badr, Abdel-Fatah, Sree, & Amra, 2017; Badr, Shehata, & Abdel-Razek, 2017). Food crops may contaminate by a load of mycotoxins (Sabry, Hathout, Nooh, Aly, & Shehata, 2016). Pizzolitto, Armando, Salvano, Dalcero, and Rosa (2013) evaluated yeast strain CECT 1891 capability as aflatoxin B1 (AFB1) reducer in the feed. It mitigated the aflatoxins effect on growth representation parameters. The study referred to yeast functionality in reducing the aflatoxicosis occurred by contaminated diet. Finally, they recommended CECT 1891 for AFB1 adsorbent.

The purpose of the present study was to evaluate the Zn-yeast capability to inhibit food hazards such as toxicogenic fungi and its metabolites like aflatoxins. As more benefit of Zn-yeast, it was applied in Fino bread production to enhance the bakery-products quality, improve the safety properties, and extended shelf life.

2. Materials and methods

2.1. Maintenance of microorganism and cultivation process

The experimental microorganisms were grown at 25°C on an orbital shaker (150 rpm) in a medium comprising (g/L): D-glucose 20.0; yeast extract 1.0; KH2PO4, 2.72; K2HPO4, 3H2O, 5.2; (NH4)2SO4, 2.0; MgSO4, 7H2O, 0.12; FeSO4, 7H2O, 0.002; ZnSO4·7H2O, 0.004; MnSO4, 4H2O, 0.004; CuSO4, 5H2O, 0.0004. Saccharomyces cerevisiae strains were routinely maintained at 25°C on a solid medium comprising (g/L) malt extract, 3.0; yeast extract, 3.0; bacteriological peptone, 5.0; D-glucose 10.0; agar 15. The yeasts strains were lyophilized for further application.

2.2. Quantitative analysis of zinc

Total cellular zinc accumulation was determined using PerkinElmer 2800 Atomic absorption spectrophotometry at growth and stationary phase (Kubaszewski et al., 2014). Concentration of zinc sulphate was adjusted at 600µg/L in media. Cells were harvested by centrifugation for 5 min at 1000 X g and washed twice in cold 1 mM EDTA, then once in cold distilled de-ionized water to remove surface-bound zinc. Cells were then digested overnight at room temperature in 5 ml 15% H2SO4, 40% HNO3. Acid-digested samples diluted with distilled de-ionized water, and zinc content was measured.

2.3. Antagonism assay between yeast NRC112 and toxicogenic fungi in solid and liquid media

Potato dextrose broth (PDB) prepared for yeast concentration of 1.18 x 10^7 CFU/mL (Fratianni et al., 2014). Six species of Aspergillus fungi, (A. parasiticus ATCC 15,517, A. flavus ATCC 9643, A. ochraceus ATCC 18,500, A. niger ATCC 10,535, A. nidulans ATCC 26,424, and A. oryzae ATCC 11,866), were applied to evaluate inhibition of fungal growth in the presence of the yeast strains. Fungi strains inoculated to sterile potato dextrose agar (PDA), using wells (5 mm) cut in the plates about 1 ml of yeast transferred to fungi inoculated plates in four wells. The antagonistic effect recorded as zone inhibition after five days of incubation.

For liquid media growth of toxicogenic fungi, a 250 ml yeast extract sucrose media (YES) prepared in one-liter conical flasks, inoculated by fungi concentration spores of 10^6 CFU/mL for each fungus strain separately (Badr, Logrieco, Amra & Hussein, 2017; Abdel-Fatah, Badr, Seif, Ali, & Hassan, 2018). Yeast broth of NRC112 added at concentration 1.18 x 10^7 CFU/mL (1 mL) to fungal media, and flasks incubated at 25°C for 5 days. The inhibition effect determined as the mycelia growth weight losses against control fungi growth.

2.4. Aflatoxins reduction by saccharomyces strain NRC112 on liquid media

In 250 ml of YES media, a concentration of A. parasiticus ATCC 15,517 spore (1.41 x 10^6 CFU/mL) inoculated in 1 L conical flask. NRC112 strain grew in 100 mL of YPD media to reach 1.18 x 10^7 CFU/mL, then added to fungi flasks, incubated 8 days/25°C, aflatoxins reducing evaluated against control flask without yeast.

2.5. Baking quality, freshness, and sensory evaluation of fino bread

Fino bread prepared according to the procedure described by (Mohamed, El-Desouky, Hussein, Mohamed, & Naguib, 2016). Weight, volume, and specific volume of Fino loaves determined as described in (A.A.C.C, 2000) methods. Loaves volume measured using rapeseeds displacement method (Collins et al., 2006). The freshness of each packed Fino estimated by alkaline water retention capacity (Mohamed et al., 2016). Moisture, ash, fibre, protein, and fat of raw materials and different Fino bread determined according to AOAC (2000).

2.6. Texture properties of fino bread

Texture parameters of Fino samples measured using a texture analyser TA-CT3 (Brookfield, USA) according to A. A.C.C (2000). The compression test selected in texture analysis using a 10 kg load cell, the sample compressed to 45% of its original height. The strain required for 45% compression was recorded using the following conditions: test speed: 5.0 mm/s, post-test speed: 2 mm/s, compression distance: 8 mm and trigger type: auto 20 g.

2.7. Fino bread time extended of shelf life

The Fino shelf-life extension was estimated to evaluate the Zn-yeast application. The experiment divided into four groups: 1) control Fino stored at 4°C; 2) control Fino stored at 25°C; 3) Zn-yeast stored at 4°C, and 4) Zn-yeast stored at 25°C. All bags have inoculated with A. parasiticus strain ATCC 15,517. The extension of shelf-life recorded as the resist to the fungi growth on Fino.

2.8. Determination of aflatoxin in samples by HPLC

Four types of Aflatoxin (B1, B2, G1, and G2) standards received as crystals: Calculated volume for Fino spiked application prepared according to Lee et al. (2003). Aflatoxins were
recovered from media or Fino samples using methanol: water (80:20 v/v) then cleaned up by Vicam™ column C18 using a peristaltic pump at flow rate of 0.8 ml/min. One hundred µl were used to HPLC injection. High-performance liquid chromatography (HPLC) system consisted of Waters Binary Pump Model 1525, Model Waters 1500 Rheodyne Manual Injector, Waters 2475 Multi-Wavelength Fluorescence Detector, and a data workstation with software Breeze LOQ (1 ng). A Phenomenex Column C18, dimensions: 250 × 4.6 mm, particle size: 5 µm, from Waters Corporation (USA) as well as Microfiber Filters, 11 cm, product ID: 31,955, VICAM Company (Sweden), were used.

2.9. Statistical analysis
The experiment was tested statistically using the ANOVA procedure SPSS 16 software. Verification of significance calculated based on P ≤ 0.05 according (Badr et al., 2017)

3. Results and discussion
3.1. Effect of different zinc salts and pH of fermentation media on yeast strain uptakes

The effect of various zinc salts (ZnCl₂, Zn SO₄, Zn CO₃ and ZnO) on the uptake process investigated. The results in Table 1 showed that by using molar solutions of several zinc salts, the kind of zinc salt significantly affected the uptake process where the best uptake (567.66 µg/g) obtained on the growth phase which had a biomass equal to 466.66 mg/100 ml using zinc sulphate with present yeast strain NRC 112. This agreed with the result of Azad, Shariatmadari, & Torshizi (2014) who has stated the priority of using zinc sulphate as the zinc source. Otherwise, using zinc oxide gives the lowest uptake due to increasing of oxidation reactions in the fermentation medium.

Zn uptake was investigated at various pH values of fermentation medium using zinc sulphate salt (Table 1), a significant effect by changes of pH has recorded. The acidic pH values found to support the uptake process whereas; the best Zn uptake (744 µg/g) obtained at pH 5.5. Meanwhile, the pH changes towards the neutral pH decrease Zn uptake rate (73.32 µg/g), this finding agreed with results of Malgorzata, Stanislaw, Joanna, and Wanda (2006) and De Nicola, Hall, Bollag, Thermodiannis, and Walker (2009).

3.2. Effect of different intact time, zinc sulphate concentration, and temperatures on the accumulation process

Different intact time has investigated as described in the Figure 1(a) for its impact on zinc uptakes, the best zinc uptake was obtained at 72 h of an intact period. In the Figure 1(b) the yeast biomass changes were evaluated against several intact times, the results monitoring the highest yeast biomass at 72 h followed by 48 h of intact time. However, by a longer fermentation time noticed a reduction in zinc uptake. Yeast effectively accumulates the essential minerals like zinc which perform a vital role. A higher zinc uptake during the growth phase was recorded compared to the stationary phase, this indicates high yeast cell ability to absorb zinc during the growth phase. Zinc has transferred from metal to organic form by an uptake process which is more available in the cell system (Smith & Walker, 2000).

The data presented in Figure 1(c) revealed that: the best zinc uptake (1623 µg/g) has obtained at 600 µg/L zinc sulphate concentration. The variation in zinc concentrations far from this value showed a remarkable decrease in the uptake process. The different levels of zinc addition have experimented, in a previous investigation, a complicated connection had been reported between cell membrane fluidity and zinc level (De Nicola et al., 2009). There is a combination for the linkage amidst yeast cell zinc status and the fluidity of the membrane, cells with low zinc content exhibit a high fluidity of the cell membrane (MacDiarmid, Gaither, & Eide, 2000).

Different temperatures effect on the uptake process had tested. The results which presented in Figure 2 showed that; the value of maximum zinc uptake (1623 µg/g) obtained at 25°C. At the higher and the lower temperatures values, the changes in the zinc uptake activity noticed. Again, the uptake of metal reversely proportional to the high temperature of the fermentation medium (Simm et al., 2007).

3.3. Inhibition effect of yeast NRC112 on toxigenic fungi strains
Antagonistic effect between NRC112 strain and six of Aspergillus fungi species has evaluated on PDA plates. Inhibition zone has recorded a greater using Zn-yeast more than control yeast. The distance between fungal growth and yeast inoculated wells displayed more clearance with Zn-yeast strain NRC 112. The clear zone recorded larger in case of A. niger ATCC 10,535. However, the smallest one represented for A. parasiticus ATCC 15,517. On liquid media of YES; the control negative applied using the fungal strain only. The Zn-yeast effect on the fungal growth under-investigated ordered ascending according to the reduced mycelia weight as A. parasiticus ATCC 15,517 < A. Flavus ATCC 9643 < A. nidulans ATCC 26,424 < A. ochraceus ATCC 18,500 < A. oryzae ATCC 11,866 < A. niger ATCC 10,535. The results declared a significant impact of Zn-yeast to inhibit toxigenic fungi growth in liquid media (Table 2).

### Table 1. Effect of Zinc salts and fermentation pH values on zinc uptakes by S. cerevisiae.

| Treatment        | Biomass (mg/100 ml) | Zinc Content (µg/g) | Biomass (mg/100 ml) | Zinc Content (µg/g) |
|------------------|---------------------|---------------------|---------------------|---------------------|
| **Different zinc salts on zinc uptake** |                      |                     |                     |                     |
| Zn Cl₂           | 356.00 A            | 443.33 B            | 255.33 A            | 347.33 A            |
| Zn SO₄           | 466.66 B            | 567.66 B            | 248.00 A            | 362.66 A            |
| ZnCO₃            | 240.33 C            | 337.33 C            | 239.00 A            | 333.33 B            |
| ZnO              | 228.66 C            | 227.33 D            | 233.33 A            | 283.66 C            |
| **fermentation pH effects on zinc uptakes** |                      |                     |                     |                     |
| 4.5              | 240.00 C            | 295.33 C            | 240.00 C            | 60.33 D             |
| 5                | 233.66 D            | 496.00 B            | 250.33 CB           | 91.33 C             |
| 5.5              | 403.66 C            | 744.00 A            | 268.67 CB           | 233.66 A            |
| 6                | 444.66 B            | 343.33 C            | 318.33 A            | 165.66 B            |
| 6.5              | 492.33 A            | 147.67 D            | 251.56 CB           | 87.66 C             |
| 7                | 435.66 B            | 73.33 E             | 280.67 B            | 87.66 C             |

- Means with same superscripts in the same column are not significantly different (P ≤ 0.05).
- The values in each cell of table calculated as a mean of triplicate values.
- Las medias con los mismos superindices en la misma columna no son significativamente diferentes (P < 0.05).
- Los valores en cada celda de la tabla fueron calculados como una media de valores por triplicado.

### Table 2. Effect of intact time, zinc sulphate concentration, and temperatures on the accumulation process.

| Treatment        | Biomass (mg/100 ml) | Zinc Content (µg/g) |
|------------------|---------------------|---------------------|
| **Different intact time** |                      |                     |
| 4.5              | 240.00 C            | 295.33 C            |
| 5                | 233.66 D            | 496.00 B            |
| 5.5              | 403.66 C            | 744.00 A            |
| 6                | 444.66 B            | 343.33 C            |
| 6.5              | 492.33 A            | 147.67 D            |
| 7                | 435.66 B            | 73.33 E             |
3.4. Organoleptic properties and baking quality of Fino

The Chemical Composition for baked Fino analytically determined. There were no significant differences between the Fino produced from control yeast or by Zn-yeast for the moisture, protein, fat, crude fiber, ash, and total carbohydrate values Table 3. The organoleptic properties of baked Fino produced by control yeast and Zn-yeast NRC 112 evaluated in Table 4. A significant difference in several characteristics such as taste, aroma, mouth-feel, crumb texture, crumb color, crust color, break & shred, and symmetry shape. Fino bread produced from Zn-yeast showed significantly higher sensory scores than those prepared with from control yeast. Furthermore; non-significant differences (at p ≤ 0.05) noted for normal Fino bread in Crumb color, Break & shred, and Symmetry shape.

The bread-volume and the specific volume of Fino manufactured by Zn-yeast recorded lower values compared to the control (Table 4). This decreases had no significant effect on the sensory as represented in sensory evaluation of Fino. The main target of the Zn-yeast application was increased the

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**Table 3**

| Moisture | Protein | Fat | Fiber | Ash | Total Carbohydrate |
|----------|---------|-----|-------|-----|-------------------|
| Control  | Zn-yeast | Control  | Zn-yeast | Control  | Zn-yeast |
| %        | %       | %   | %     | %   | %                |
| 12.3     | 12.4    | 12.1 | 12.2  | 12.0 | 12.1             |

**Table 4**

| Characteristics | Control Yeast | Zn-Yeast NRC 112 |
|-----------------|---------------|------------------|
| Taste           | 7.5           | 8.0              |
| Aroma           | 7.2           | 7.4              |
| Mouth-feel      | 7.0           | 7.2              |
| Crumb Texture   | 7.3           | 7.6              |
| Crumb Color     | 7.1           | 7.3              |
| Crust Color     | 6.9           | 7.2              |
| Break & Shred   | 7.4           | 7.6              |
| Symmetry Shape  | 7.0           | 7.2              |

**Figure 1.** Time intact and Zn So₄ concentration effect on zinc uptake by yeast strain NRC112

![Figure 1](image)

- **A**: effect of intact time on zinc uptake; **B**: effect of intact time on yeast biomass; **C**: effect of ZnSo₄ concentrations on Zn-uptake. The values were calculated as a mean of triplicate values (P ≤ 0.05), the treatment showed a significance of differences.

- **A**: efecto del tiempo intacto en la absorción de zinc; **B**: efecto del tiempo intacto en la biomasa de levadura; **C**: efecto de las concentraciones de ZnSo₄ en la absorción de Zn. Los valores se calcularon como una media de los valores por triplicado (P < 0.05); el tratamiento mostró que las diferencias encontradas son significativas.
Effect of zinc concentration on the uptake and biomass of yeast

Figure 2. Effect of different temperature on the uptake of zinc

Figura 2. Efecto de diferentes temperaturas en la absorción de zinc

- The values were calculated as a mean of triplicate values (P ≤ 0.05), the treatment showed a significance of differences.
- Los valores se calcularon como una media de los valores por triplicado (P < 0.05); el tratamiento mostró que las diferencias encontradas son significativas.

Table 2. Mycelial weight Inhibition and diameter of inhibition zone of toxigenic fungi in a presence of yeast strains.

| Toxigenic Fungi strain          | Reduction of mycelia | Zone inhibition diameter |
|--------------------------------|-----------------------|--------------------------|
|                                | Non-rich Saccharomyces| Saccharomyces enriched by Zn | Non-rich Saccharomyces | Saccharomyces enriched by Zn |
|                                | % inhibition          | % inhibition          | Inhibition (mm)       | Inhibition (mm)       |
| Aspergillus parasiticus        | 60.82 ± 2.12          | 73.4 ± 2.48             | 7.16 ± 3.11           | 12.89 ± 3.57          |
| ATCC 15,517                    |                       |                         |                        |                        |
| Aspergillus flavus             | 71.93 ± 2.39          | 77.55 ± 3.11            | 7.65 ± 2.95           | 13.77 ± 3.91          |
| ATCC 9643                      |                       |                         |                        |                        |
| Aspergillus ochraceus          | 75.9 ± 2.48           | 79.75 ± 2.96            | 7.44 ± 1.97           | 13.59 ± 5.41          |
| ATCC 18,500                    |                       |                         |                        |                        |
| Aspergillus niger              | 81.03 ± 3.44          | 96.13 ± 2.57            | 8.29 ± 2.34           | 15.46 ± 2.94          |
| ATCC 10,535                    |                       |                         |                        |                        |
| Aspergillus nidulans           | 72.46 ± 2.34          | 78.13 ± 2.41            | 7.39 ± 3.54           | 13.65 ± 3.66          |
| ATCC 26,424                    |                       |                         |                        |                        |
| Aspergillus oryzae             | 76.26 ± 2.59          | 79.87 ± 2.55            | 7.27 ± 1.4            | 15.33 ± 1.4           |
| ATCC11866                      |                       |                         |                        |                        |

- Mycelial weight Inhibition evaluated on liquid media; inhibition zone diameter estimated on agar media.
- Values are presented as means ± S.D; values with same letter are non-significant (n = 5; P ≤ 0.05).
- Inhibición del peso micelial evaluada en medios líquidos; diámetro de la zona de inhibición estimado en medios de agar.
- Los valores se presentan como medias ± DE; los valores con la misma letra no son significativos (n = 5; P ≤ 0.05).

Product safety and shelf-life extension so the few changes (without sensory refused) could be accepted. Goldstein and Seetharaman (2016) were reported a great relation between loaf specific volume and bread staling. Also, the bread specific volume has correlated to changes happened in crumb elastic modulus. This relation refers to the Zn-yeast function in enhancing Fino organoleptic properties.

3.5. Freshness of Fino bread

The effect of room temperature storage on the freshness of Fino bread evaluated in Table 2. The Fino produced by Zn-yeast had the highest values of AWRC which declined during 0, 24, 48 and 72 hrs of storage. However, control Fino caused a noticeable decrease in alkaline water retention capacity at the same storage period.
Table 3. Chemical composition of control and Zn-yeast Fino bread.

| Samples                      | Moisture (%) | Protein (%) | Fat (%) | Fibre (%) | Ash (%) | Total carbohydrate (%) |
|------------------------------|--------------|-------------|---------|-----------|---------|------------------------|
| Fino bread from yeast without zinc | 35.89 ± 0.19 | 10.72 ± 0.09 | 1.95 ± 0.03 | 0.64 ± 0.03 | 1.15 ± 0.02 | 85.54 ± 0.58 |
| Fino bread from yeast with zinc    | 35.52 ± 0.15 | 10.65 ± 0.11 | 1.82 ± 0.05 | 0.69 ± 0.01 | 1.10 ± 0.01 | 89.74 ± 0.65 |
| LSD at 0.05                   | NS           | NS          | NS      | NS        | NS      | NS                     |

- The values with same letter are non-significant (P < 0.05).
- The values in each cell of table calculated as a mean of triplicate values
- Los valores con la misma letra no son significativos (P < 0.05).
- Los valores en cada celda de la tabla fueron calculados como una media de valores por triplicado.

Table 4. Organoleptic characteristics and baking quality of Fino manufactured from non-Zn and Zn-yeast.

| Samples                      | Taste (20) | Aroma (20) | Mouth feel (10) | Crumb texture (15) | Crumb color (10) | Crust color (10) | Break and shred (10) | Symmetry shape (5) | Weight (g) | Volume (cm3) | Specific volume (cm3/g) |
|------------------------------|------------|------------|-----------------|--------------------|------------------|------------------|---------------------|---------------------|------------|-------------|------------------------|
| Fino from Zn-yeast           | 18.60°     | 18.33°     | 9.10°           | 14.23°             | 9.20°            | 9.15°            | 9.20°               | 4.40°               | 134.75°    | 430.00°     | 3.19°                  |
| Fino from non Zn-yeast       | 17.50°     | 17.50°     | 8.50°           | 13.60°             | 9.15°            | 8.85°            | 9.12°               | 4.50°               | 133.75°    | 499.00°     | 3.73°                  |
| LSD at 0.05                  | 0.585      | 0.481      | 0.321           | 0.444              | 0.225            | 0.251            | 0.240               | 0.195               | 4.415      | 9.844       | 0.118                  |

- The values with same letter are non-significant (P < 0.05).
- The values in each cell of table calculated as a mean of triplicate values
- Los valores con la misma letra no son significativos (P < 0.05).
- Los valores en cada celda de la tabla fueron calculados como una media de valores por triplicado.

Table 5. Effect of different yeast types on the freshness properties of Fino during storage period.

| Samples                      | Storage time (hrs.) | AWRC at zero time | After 24hrs. | After 48hrs. | After 72hrs. | AWRC at zero time | After 24hrs. | After 48hrs. | After 72hrs. | AWRC at zero time | After 24hrs. | After 48hrs. | After 72hrs. | AWRC at zero time | After 24hrs. | After 48hrs. | After 72hrs. | AWRC at zero time | After 24hrs. | After 48hrs. | After 72hrs. |
|------------------------------|---------------------|-------------------|-------------|-------------|-------------|-------------------|-------------|-------------|-------------|-------------------|-------------|-------------|-------------|-------------------|-------------|-------------|-------------|-------------------|-------------|-------------|-------------|
| Fino from Zn-yeast           | 321°                | 280°              | 12.77°      | 244°        | 244°        | 236°              | 264°        | 46°         | 48°         | 236°              | 264°        | 46°         | 48°         | 236°              | 264°        | 46°         | 48°         |
| Fino from non Zn-yeast       | 318°                | 278°              | 12.57°      | 257°        | 257°        | 247°              | 223°        | 50°         | 50°         | 247°              | 223°        | 50°         | 50°         | 247°              | 223°        | 50°         | 50°         |
| LSD at 0.05                  | 5.186               | 4.879             | 0.541       | 6.546       | 2.170        | 7.045             | 2.681       |             |             |                   |             |             |             |                   |             |             |             |

- The values with same letter are non-significant (P < 0.05).
- The values in each cell of table calculated as a mean of triplicate values
- Los valores con la misma letra no son significativos (P < 0.05).
- Los valores en cada celda de la tabla fueron calculados como una media de valores por triplicado.

3.6. Textural properties and color attributes of backed fino

Texture profile analysis (TPA) is a benefit technique investigating physical food properties. TPA parameters for Zn-yeast Fino and control-yeast Fino evaluated by texture analyser on double compression tests. Hardness, springiness, cohesiveness, chewiness and gumminess values for control Fino had no significant differences among Zn-yeast Fino. This result indicates that Zn-yeast application, instead of control yeast, in Fino manufacturing eligible saving the Fino quality parameters without alters. Color is a sensory attribute affect directly the consumer preference. The Fino estimated colour evaluated by the Hunter laboratory colorimeter Results noted no significant differences between the Zn-yeast Fino and the control Fino (Table 6).

3.7. Reducing of aflatoxins in the presence of yeast NRC112

The traditional Saccharomyces strain and Zn-yeast strain effect for aflatoxins reducing, from YES media, compared to the control (fungi only) evaluated using HPLC technique. According to results recorded in Table 7; both yeast strains have a capability for aflatoxin reducing in media, Fino paste, and Fino bread. Inhibition effect of Zn-yeast strain was reported more effective to reduce aflatoxins either in liquid media and Fino paste. The
control treatment in YES media was the toxigenic fungi strain of *A. parasiticus* ATCC 15,517 which excreted aflatoxin B$_1$, aflatoxin B$_2$, aflatoxin G$_1$, and aflatoxin G$_2$ at the concentration of 200 ± 1.1, 120 ± 3.6, 180 ± 2.7, and 90 ± 1.4, respectively. In case of the control of Fino paste or bread, all components added to the YES media except the yeast strain. The results indicated that; Zn-yeast supported the efficient in aflatoxins decreases from liquid media, Fino paste, and Fino bread.

### 3.8. Impact of Zn-supplement yeast on the shelf life extended

In two groups, the Fino bread storage conditions varied (at room temperature and refrigeration). The shelf life of the bread loaves calculated. The time before the fungal growth appeared evaluated, as well as, the total count of the fungal colony recorded each day to represent the bread resistance for fungal contamination. The results showed that; the bread was showed more stable against fungal growth in the case of Zn-yeast Fino than traditional yeast-Fino. The data reported a decrease in fungal growth rate on Zn-loves Fino product. Results in Figure 3, represent the variation in mold occurrence on a storing packed Fino.

![Figure 3](image)

**Figure 3.** The increase of Fino bread shelf-life using Zn-supplemented yeast

**Tabla 7.** Efecto de la levadura-Zn en la reducción de aflatoxinas de los hongos de *A. parasiticus* en medios líquidos.

| Aflatoxins | Zn-enriched yeast | Traditional yeast |
|------------|-------------------|-------------------|
| **in liquid media ng/kg** | **in Fino paste ng/kg** | **in Fino bread ng/kg** |
| AFB$_1$ | 91 ± 1.3 | 97 ± 1.7 | 200 ± 1.1 |
| AFB$_2$ | 51 ± 3.1 | 62 ± 2.3 | 120 ± 3.6 |
| AFG$_1$ | 40 ± 2.5 | 56 ± 1.4 | 180 ± 2.7 |
| AFG$_2$ | 33 ± 1.9 | 41 ± 2.2 | 90 ± 1.4 |

**Table 7.** Effect of Zn-yeast on aflatoxins reducing of *A. parasiticus* fungi on liquid media.

- Values are presented as means ±S.D (P ≤ 0.05).
- The values in each cell of table calculated as a mean of triplicate values.
- Los valores se presentan como medios ± DE (P ≤ 0.05).
- Los valores en cada celda de la tabla fueron calculados como una media de valores por triplicado.
In the present study, the optimum condition for Zn-yeast production was determined, which could lead the process of commercial manufacturing. The most benefit of the Zn-yeast application is offering zinc in organic form which is more efficient in biological process and metabolism. Not only Zn-yeast allows organic zinc in food, but also it increases the binding ability of food contaminants. The increased binding ratio of aflatoxins by Zn-yeast strain ameliorated food safety production. Furthermore, Zn-yeast NRC112 recorded a capability affect toxicogenic fungi growth. For the solid and liquid growth media of fungi: Zn-yeast had more ability to reduce the fungal growth compared to the traditional yeast strain.

Notwithstanding; some of baking quality parameters recorded a significant change (volume & specific volume) but sensory still in the acceptable range according to the evaluation test. The changes also recorded for AWRC values particularly after 24 hours of storage for Fino manufactured using Zn-yeast strain. As this work targeted to increase the safety of bakery products, also to extend its shelf-life and delay the fungal contamination. In this case, these changes can be overlooked according to the acceptance of consumers which recorded in Table 4.

Again, the application of the Zn-yeast in the YES media contains aflatoxins producing strain A. parasiticus ATCC 15,517 reported a rising or elevating of S. cerevisiae NRC 112 ability to decrease aflatoxins secretion in the growth media, this could nominate it to applied in biocontrol systems of toxigenic fungi. Comparing to the control positive (traditional S. cerevisiae) and the control negative (A. parasiticus ATCC 15,517 without yeast), the Zn-yeast recorded the best results for aflatoxin reducing. Aflatoxins reduced due to the yeast activities, also, this reducing value was increased in the presence of zinc (Zn-yeast).

The application of Zn-yeast in Fino manufacturing was reflected a more ability to resist the fungal contamination on the product. The fungal contamination as colony forming unit per gram (CFU/g) on Fino recorded lower in a case using Zn-yeast either it was stored at room temperature or refrigerated. Fino was recorded enhancing sensory properties such as the volume and specific-volume of Fino which appreciated delaying staling properties. Moreover, the organoleptic characteristics of the final product kept without altering. Supporting of bakery products may consider a novel application for food nutrition enhancement, as a method fight the malnutrition related to the zinc-lack sufficient, Zn-yeast may involve in food product particularly in children food and other sensitive scales of the human. Zn-yeast, which recorded the ability to reduce aflatoxins from media, paste, and food product could also apply in feed material which is more contaminant by aflatoxins. Zn-yeast application in the feed will be an indirect method to decrease the contamination in animal food.

4. Conclusion

The S. cerevisiae zinc uptakes influenced by zinc-salt type and the changes in fermented media parameters (pH, temperature, Zn-salt concentration, and time intact). Strain NRC112 recorded good abilities reducing toxigenic fungi growth and aflatoxins excretion in liquid media. Its application in Fino manifested enhancing of sensory scores, Baking quality, texture analysis, and the freshness of Fino produced by Zn-yeast show better properties than the control. Moreover, the application of Zn-yeast in Fino manufacturing recorded reducing of aflatoxins contamination and delay the fungi present on the final product at room temperature also on refrigeration stored condition. Fungi contamination delay declared as a shelf-life extension in the food product.

Disclosure statement
No potential conflict of interest was reported by the authors.

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FEMS
A. parasiticus
ATCC 15,517 without yeast), the Zn-yeast showed capability to reduce aflatoxins secretion in the growth media, which recorded in
170
by Zn-yeast show better properties than the control.

Moreover, the application of Zn-yeast in Fino manufacturing recorded reducing of aflatoxins contamination and delay the fungi present on the final product at room temperature also on refrigeration stored condition. Fungi contamination delay declared as a shelf-life extension in the food product.

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