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BIODEGRADATION OF RECALCITRANT FOODBORNE CLONES OF *CANDIDA ALBICANS* BY HURDLING WITH PASTEURIZATION POSTED BY PEPPERMINT PROCESSING ECOSYSTEM IN BAGHDAD, IRAQ

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Abstract

Sophisticated hygienic problems of residual-persistent thermoduric infectious foci of recalcitrant encapsulated clones of *Candida albicans* within matrix of biofilm with other dangerous microbiome causing serious multidrug resistance risks and an emerging hazard life threatening problems. These struggling could be terminated by verified hurdle biodegradation technology using pasteurization regimes cascaded by peppermint extract processing as flavoring antimicrobial potentiation synergistic module ecosystem against these forbidden survivors with superior importance to reducing and even killing regime in Baghdad. Forbidden thermoduric clones of biofilm-producing and multidrug-resistant (azoles and polyenes resistant) *C. albicans* were recovered from different dairy series ecosystems from scanned zones of Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya in Baghdad within specified episodes. Low temperature long time pasteurization module at 63°C for 30 minutes (LTLT) and high temperature short time pasteurization module at 72°C for 16 seconds (HTST) posted by processing with alcoholic and watery extracts of peppermint as a potentiation module were dependent for this enrollment strategy. Thermal reduction time (D-values) and thermal reduction temperature (Z-values) verified with the determination of lowest or minimum inhibitory and maximum fungicidal concentrations or titers of both extracts. Variable and diverse results on viability of different clones or electrotypes of *C. albicans* achieved during exposure to cascaded and verified stress-processing hygienic regimes. Watery extracts of peppermint were the powerful antimicrobial and antifungal processing in which, it diminish or reduce and even kill powerful isolates that own complex biofilm clouds, multidrug resistant and most heat tolerant thermoduric strains that not exposed earlier to thermal shock. Combined hurdle processing regimes with thermal processing cascaded by peppermint were efficient for reducing and combating the denominator to the limits that not harmful while pasteurization regimes alone could reduce majority but not thermoduric strains.

Key words: Biodegradation, *Candida albicans*, Multidrug-Resistance, Hurdle Processing, Pasteurization, Peppermint.

Introduction

Food products contamination – pollution circles inside ecosystems can be inhibited by various means: these include reduction in water activity (aw), low temperature, reduction of pH, addition of competitive microorganisms and addition of preservatives. Combinations of these various means can be used. The use of combinations is called "hurdle effect" (Leistner, 1994). Intelligent use of hurdles in food product design insures that products have an adequate shelf life and remain safe (Leistner, 1994a). Hurdle Technology is a technology by which a preservation parameter can be used at an optimum level in order to get a maximum lethality against micro-organisms by a combination of two or more such parameters so that the damage to the sensory parameters of the food is kept to the minimum (Singh and Shalini, 2017). Farnesol-Tyrosol Quorum sensing behavior is the brain-like machine in forbidden clones of *C. albicans* that orchestrate and remodeling their homeostasis throughout stress-adaptation and stress-hardening cascaded phenomenon as pheromone signal that sense environment and transfer their neuron-like impulses throughout sophisticated manner across complex and hidden nano bionetwork in order to regulate their switching morphogenesis, germ tube, biofilm formation, virulence biomarkers and multidrug resistance (Rodrigues and Cernakova, 2020).

Medicinal plants have been used for centuries in traditional medicine because of their therapeutic value. Mint species have been exploited by man for more than two thousand years, which has an economical value for

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its flavoring, odor, and therapeutic properties in foods and cosmetic industrial products. Peppermint (*M. piperita*) oil is one of the most popular and widely used essential oils, mostly because of its main components Menthol and menthone. Previous studies have shown antiviral, antibacterial, antifungal, anti-biofilm formation, radio protective, antioedema, analgesic and antioxidant activities of the EO and methanolic extracts of herbal parts and callus cultures of *M. piperita*. In addition, *M. piperita* EO has been shown to cause inhibitory effects against radial fungal growth and aflatoxin production by *Aspergillus* species. In the past two decades, the emergence of resistance to various antifungal drugs has accelerated dramatically. Azole-resistant *Candida* and *Aspergillus* species are the top pathogens responsible for nosocomial or foodborne infections. In addition, the formation of biofilms by *Candida* species have raised concerns due to their increased resistance to antifungal therapy and protects the microbial cells within biofilms from the host immune defenses (Saharkhiz *et al*., 2012). Previous study unveiled the effect of different concentrations of hydro alcoholic leaves extract of *Datura stramonium* against pathogenic *C. albicans* isolated from clinical cases of diarrhea in cows and dogs in Baghdad (Salman and Faraj, 2015).

Cascaded series of hygienic and flavoring processing ecosystems unveiled by pasteurization regimes and fresh peppermint extracts against recalcitrant infectious foci of foodborne clones of *C. albicans* recovered from dairy products in Baghdad was the mainstream focus of this verified project about multidrug-resistance problems.

Materials and Methods

Primordial Recovery: *C. albicans* clones recovered from dairy series ecosystems of Cows raw milk, fresh-soured yogurt, fresh-brined soft cheese-whey and butter-cream pooled samples from regions of Abu-Ghraib, Al-Fudhaliyah and Al-Sadrya within specified periods. HiCrome Candida selective-differential agar was dependent for this enrollment. Modified Congo red agar was dependent for assaying and segregation of isolates into biofilm-producing and plasmid equipping strains. HiMedia (2019) dependent antifungals susceptibility kits including Radial Sensititer Hexa Antimyco-01 (HX104) and Fluconazole Ezy MICTM Sensititer Strip (FLC Epsilometer (E test) (EM072) (0.016-256 mcg/ml) were segregate clones into azoles-polyenes resistant, intermediate and sensitive. Resistant strains authorized by Supervisor (Al-Shammary) to experimentally designed hurdle processing with pasteurization (LTLT and HTST) potentiated by watery and alcoholic extracts of fresh peppermint.

Hurdle Processing Scheme: Verified cascaded series of processing *in vitro* models inside experimentally created environment for induced contamination of either modified serum TSB-YE interphase or naturally fungal-free imported tetra pack KDD milk. McFarland turbidity spectrophotometric assay enrolled with modified droplet technique and modified rolling cap-pour plate technique supports standardized freshly prepared nine log (10^9 CFU.ml^{-1}) of resistant chosen C. *albicans* clones to be the line of demarcation for all single, double and cascaded experiments. Cascaded processing series enrolled in watery or alcoholic extracts efficacy regime versus dual thermal processing with either low temperature long time pasteurization or high temperature short time pasteurization. Combination Bio Preservation potentiation module with peppermint extracts followed by thermal pasteurization or *vice versa* pasteurization followed by treatments with peppermint extracts cascaded with other natural cooling inside a refrigerator so called hurdle-processing hazard analysis critical control points (HACCP) strategies employed for controlling or reducing experimentally contaminated media or milk with verified log count of ARCA *C. albicans*. Reciprocal vice versa processing techniques were dependent in which, pasteurization decorum firstly enrolled at 63°C for 30 minutes and 72°C for 16 seconds for induced contaminated milk samples with standardized log titer of ARCA chosen *C. albicans*. Cooling one hour of thermally processed decorum then adding 1 % of either extracts to them as overhead upstairs formulas, vortex and homogenization proceeds with incubation at 4 °C for one-hour cascade by counting profile index as above directly and after 24 and 48 hours inside a refrigerator for same equivocal etiology.

Peppermint Extraction: Preparation of crude concentrated and diluted double distillated or purified extracts of locally fresh collected peppermint leaves and stalks from different regions in Baghdad as watery and oily or alcoholic extracts according to recommendations of Codex international standards of Association of Official Agricultural Chemists (AOAC) (2016).

Extracts Disc-Diffusion Susceptibility Test: Preparation of extract impregnated discs with comparison to Kirby antifungal disc diffusion assay was carried out (CLSI, 2009 and 2011).

Biofilm inhibition assay: Reciprocal vice versa *in vitro* reverse biofilm formation assay (Christensen *et al*., 1985) was dependent as a motile force checkerboard designed technique for checking the degree of success

treatments or processing with one decorum or hurdle score. Post-processing residuals enter this cycle. So that reducing or preventing biofilm-slim congelation during growth curve stages and stopping or delaying i.e. extending lag phase of resistant clones could be helpful strategy for rendering this transformed yeast to be sensitive again to traditionally used antifungal agents. Otherwise, these ruminants' residual tolerant persisters could be harsh more and transferred to super resistant clones or so-called ancient ancestors of chimeras. In the same time, another set of unprocessed ARCA clones not subjected to pasteurization or hurdle regime were checked up for their ability and capacity to produce biofilm inside microtiter plates of biofilm assay after treatment with concentrated extracts of peppermint. Culturing microtiter plate's filled-holes with 5 ml double strengthen power TSB-YE inoculated with 0.1 ml McFarland 5 log titer of *C. albicans* then adding 0.1 ml concentrated extracts from both phases in separate plates. Control negative holes with only extracts or PBS versus control positive holes with *C. albicans* in the same tray system were dependent for guided comparisons. Same as the procedure of biofilm assay, the microtiter plates were processed with dual sensitive staining technique with dual incubation at 25 and 37°C for (18-24) hours to refining the observed results. Biofilm anti-biofilm sequesters were checked in this checkerboard experienced designed trait.

Statistical analysis: Bio statistically dependent software of Statistical Package for the Social Sciences (SPSS, version 27, 2020), including t-test and Chi-square for scan disparities among data.

Results and Discussion

Variable and diverse results on viability of different clones or electrotypes of *C. albicans* achieved during exposure to cascaded and verified stress-processing hygienic regimes. Watery extracts of peppermint were the powerful antimicrobial and antifungal processing in which, it diminish or reduce and even kill powerful isolates that own complex biofilm clouds, multidrug resistant and most heat tolerant thermoduric strains that not exposed earlier to thermal shock. This finding was wonderful due to availability of low cost plant as well as easiest household preparation of watery extract (tincture) or even consumption of fresh plant without processing will assist in superior combating the foodborne-targeted clones of *C. albicans* lineage. Combined hurdle processing regimes with thermal processing cascaded by peppermint were efficient for reducing the pathogen to the limits that not harmful while pasteurization regimes alone could reduce majority but not thermoduric strains. Thermal processing could sense something in genetic material of some clones throughout quorum sensing stress-adaptation or stress-hardening machine that induced remodeling of their cell membrane or cell wall with refolding of heat shock proteins terminating with the regeneration of new clones owns broad-spectrum genetic elasticity that resist all lethal hygienic treatments even with peppermint that not exposed to it in their lifestyle.

According to Supervisor authorized experimental design of single peppermint plus thermal pasteurization plus Hurdle technology, only selected and verified azoles resistant clones of *C. albicans* were scheduled experiments proceeds. Thermal reduction time (D values) and thermal reduction temperature (Z values) was calculated to achieve approximately natural real observed time and temperature total mean value needed for a better reduction or elimination of these pathogenic clones from contaminated milk. To obtain information about the number of cells surviving after different periods of heating, this logarithmic equation can be integrated between time zero, time t, temperature zero, and temperature *T* (Adams and Moss, 2008).

These equations builds Bio statistically on correlation-regression relationships linear models between linked variables, so when the D or Z values were negative with increase or decrease in numbers of calculated logs rhythm, that means reciprocal integration between time and decimal counting log of *C. albicans* verified clone versus reciprocal integration between temperature and decimal counting log of *C. albicans* verified clone. In other words, when exposing time increase during thermal processing like LTLT or when exposing temperature was higher during thermal processing like HTST reciprocal integrated with thermal reduction of contaminated *C. albicans* verified clone by one decimal log after thermal processing interpretation as increasing negative scores of D and Z values i.e. good hygienic result processing treatments and strong correlation with the increase negative score or slope of *C. albicans* verified clone. According to Jay formula in food microbiology (2005) each 0.5 log decrease or increase in mean log count reflect the sensitivity and specificity ratios of that processing, *i.e.* the real amount of calculated cross-tabulated minus logs of true and false positives coherent pairs versus true and false negative coherent pairs with standard errors in receiver operating characteristic model. Tables (1-6) illustrate LTLT and HTST processing with deciphered Fig. 1.

Combination of two or more hygienic processing represent modern technology in food processing regimes that assist in functional bio-preservation of food with integrated synergistic efficacy modules that protect

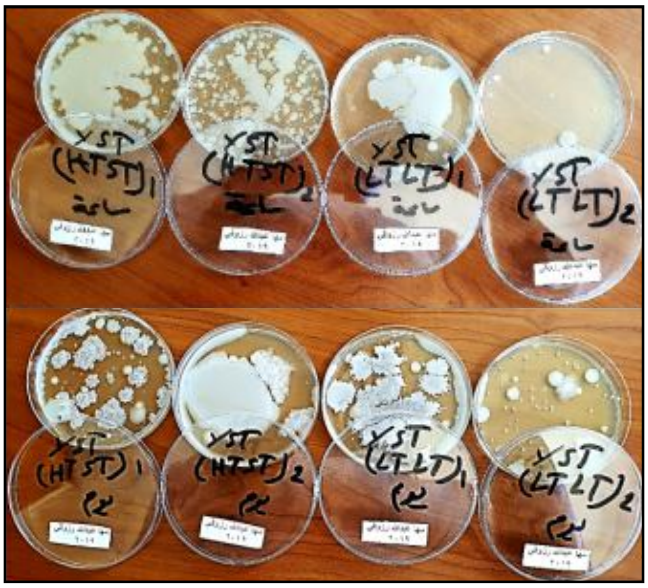

Fig. 1: Deciphering thermal processing (LTLT and HTST) of milk contaminated with *C. albicans* clones lineage.

organoleptic components throughout reducing contaminant microbial (opportunistic pathogens) load logs with their spoilage enzymes and toxins as well as, increase shelf life of food, *i.e.* extend lag phase of pathogenic contaminants. Peppermint considered as powerful antimicrobial agent and flavoring prebiotic component that enhance taste and odour of food and act as powerful nutrient suppliers for beneficial probiotic microflora and immune potentiator for macrophage-neutrophils lineage defense barrier. Diverse and versatile results during treatments of induced contaminant of milk with six fold logs of *C. albicans* clones. Pre Pasteurization regime followed by post-pasteurization flavoring enrichment with peppermint extracts or *vice versa*, enrollment with peppermint extracts cascaded by thermal processing throughput pasteurization regimes. In both cases, cooling module at 4°C inside a refrigerator considered third processing unit in hurdle regime as a part of hazard analysis critical control points (HACCP) hygienic protocol. Tables (7-10) illustrate Residual fold reducing logs after processing with pasteurization posted by peppermint extracts and *vice versa* hurdling regime with Fig. 2 and 3 display efficacy behavior of combo dual processing.

Variable and strange interconnected residual persisters or sublethal damaged forbidden clones and sub clones resuscitated during cooling preservation inside a refrigerator with proliferating dangerous profile and behavior. Diverse and versatile thermotolerant-thermoduric survivors displayed during achievement of thermal processing at LTLT module versus HTST module. Some highly dangerous forbidden clone persists, resists pasteurization at LTLT module, and even proliferate after settling overnight at refrigerator. These thermos entities recovered from raw milk, fresh yogurt, fresh soft cheese-whey and cream ecosystems in Al-Fudhaliyah, from raw milk, fresh-brined soft cheese-whey and cream ecosystems in Abu-Ghraib and from raw milk, fresh yogurt-soft cheese-whey and cream ecosystems in Al-Sadrya. Similar forbidden findings presents during HTST achievement in which, semiconservative thermos-forbidden clones displayed from all dairy brands in all scanned zones. These forbidden behaviors refers to quorum sensing with changing in homeostasis, i.e. shifting or switching behavior due to stress hardening and balloon plasticity from normal turnover state to straggling activated module especially in HTST module and that verified cue ensure that the time factor in pasteurization module (D value) was valuable versus temperature module (Z value).

Table 2: Viability of Abu-Ghraib ARCA clones growth curve or generation time after pasteurization of induced contamination UHT milk units at 63°C for 30 minutes (LTLT).

ARCA clone ancestor	Mean Log Count CFU.ml^{-1} after LTLT processing		
	McFarland Control Log (Time Zero)	One hour settling at lab. Temperature	Twenty-four hours settling at 4°C
Raw Milk	10^6 CFU.ml^{-1} or 6 logs Standardized	6 Logs	2-4 Logs
Fresh Yogurt		1-2 Logs	0-2 Logs
Soured Yogurt		1-2 Logs	0-1 Logs
Fresh Soft-Cheese		3-5 Logs	6 Logs
Brined Soft-Cheese		3-6 Logs	6 Logs
Butter		1-2 Logs	0-1 Logs
Cream		3-4 Logs	3-4 Logs

Table 3: Viability of Al-Sadrya ARCA clones growth curve or generation time after pasteurization of induced contamination UHT milk units at 63°C for 30 minutes (LTLT).

ARCA clone ancestor	Mean Log Count CFU.ml^{-1} after LTLT processing		
	McFarland Control Log (Time Zero)	One hour settling at lab. Temperature	Twenty-four hours settling at 4°C
Raw Milk	10^6 CFU.ml^{-1} or 6 logs Standardized	6 Logs	2-6 Logs
Fresh Yogurt		2-3 Logs	4-5 Logs
Soured Yogurt		1-3 Logs	0-1 Logs
Fresh Soft-Cheese		4 Logs	6 Logs
Brined Soft-Cheese		4 Logs	2 Logs
Butter		2 Logs	0-2 Logs
Cream		4 Logs	5 Logs

Table 1: Viability of Al-Fudhaliyah ARCA clones growth curve or generation time after pasteurization of induced contamination UHT milk units at 63°C for 30 minutes (LTLT).

ARCA clone ancestor	Mean Log Count CFU.ml^{-1} after LTLT processing		
	McFarland Control Log (Time Zero)	One hour settling at lab. Temperature	Twenty-four hours settling at 4°C
Raw Milk	10^6 CFU.ml^{-1} or 6 logs Standardized	6-8 Logs	2-6 Logs
Fresh Yogurt		2-7 Logs	2-4 Logs
Soured Yogurt		1-2 Logs	1-2 Logs
Fresh Soft-Cheese		3-5 Logs	6 Logs
Brined Soft-Cheese		4-5 Logs	2 Logs
Butter		1-2 Logs	0-2 Logs
Cream		3-5 Logs	5-6 Logs

Table 4: Viability of Al-Fudhaliyah ARCA clones growth curve or generation time after pasteurization of induced contamination UHT milk units at 72°C for 16 seconds (HTST).

ARCA clone ancestor	Mean Log Count CFU.ml^{-1} after LTLT processing		
	McFarland Control Log (Time Zero)	One hour settling at lab. Temperature	Twenty-four hours settling at 4°C
Raw Milk	10^6 CFU.ml^{-1} or 6 logs Standardized	6-7 Logs	5-7 Logs
Fresh Yogurt		2-5 Logs	1-5 Logs
Soured Yogurt		3-4 Logs	1-3 Logs
Fresh Soft-Cheese		1-6 Logs	0-4 Logs
Brined Soft-Cheese		1-6 Logs	2-6 Logs
Butter		3-6 Logs	4-6 Logs
Cream		1-6 Logs	4-6 Logs

Table 5: Viability of Abu-Ghraib ARCA clones growth curve or generation time after pasteurization of induced contamination UHT milk units at 72°C for 16 seconds (HTST).

ARCA clone ancestor	Mean Log Count CFU.ml^{-1} after LTLT processing		
	McFarland Control Log (Time Zero)	One hour settling at lab. Temperature	Twenty-four hours settling at 4°C
Raw Milk	10^6 CFU.ml^{-1} or 6 logs Standardized	6 Logs	6 Logs
Fresh Yogurt		3-5 Logs	2-5 Logs
Soured Yogurt		3-4 Logs	1-3 Logs
Fresh Soft-Cheese		1-2 Logs	0-2 Logs
Brined Soft-Cheese		1-4 Logs	2-4 Logs
Butter		1-4 Logs	2-4 Logs
Cream		1-4 Logs	4-6 Logs

These findings were of superior hazard signals especially in imported dairy products that processed by UHT module and in cases of post pasteurization contamination. According to supervisor experience thermal stress, elicit hidden sophisticated strategies inside some forbidden strains leading to submerging of inter and intra sub clones that tolerate such triggering module as well as presence of mixed foreign and learned forbidden denominators that cross protect each other.

Heat shock proteins-tolerance behavior perceived during processing of experimentally contaminated milk with recovered clones. Reciprocal-reversible and irreversible damage and reduction in food microbial log load counts or titer was achieved by exposing milk to thermal processing either low temperature 63°C for long time 30 minutes or high temperature 72°C for short time 16 seconds depending on organoleptic properties of milk types used, and hygienic environment and measurements of milk producing animals and vehicles used for processing of milk until shipment to markets. This dangerous and serious recaptured information refers to thermal tolerance that exceed thermal durable power of normal flora of pasteurized processed milk and other dairy products. Total information above refer to efficiency of thermal processing to reducing the mean log count of these resistant-sensitive clones bellow log 6 per ml or g of food with exception of low density but highly virulent clones to tolerate LTLT or HTST processing temporarily for a periods of time partially due to CRISPR-CAS strategy. These thermotolerant clones show elongated coccobacilli bodies when stained with gram stain, thick capsule when stained with India ink and profuse biofilm with discoloration-pigmentation phenomenon during Antibiogram experiment. This could be interpreted partially according to Balloon theory and Stress-Hardening phenomenon (Rowan and Anderson, 1998; Al-Shammary, 2009 and 2019; Alyais, 2019) in which, direct exposure suddenly to high temperatures for a certain period of time led to the inability to manufacture and secretion of heat-resistant protein systems (thermal shock proteins HSPs) and then their death where a continuous expansion, rapid and sudden in the exposed bacterial bodies until exploded as a result of the sudden rise in temperature like when blowing a balloon.

They did not have sufficient genetic elasticity or resilience to resist these high thermal ranges, i.e. there was insufficient time for gradual sensing of heat and thus to activate the manufacture and secretion of these proteins due to the complete, rapid and sudden inhibition of the thermal regulatory genes, which inhibited the thermal sensory system (Quorum Sense) can be used to

Table 6: Viability of Al-Sadrya ARCA clones growth curve or generation time after pasteurization of induced contamination UHT milk units at 72°C for 16 seconds (HTST).

ARCA clone ancestor	Mean Log Count CFU.ml^{-1} after LTLT processing		
	McFarland Control Log (Time Zero)	One hour settling at lab. Temperature	Twenty-four hours settling at 4°C
Raw Milk	10^6 CFU.ml^{-1} or 6 logs Standardized	6 Logs	6 Logs
Fresh Yogurt		5-7 Logs	6-8 Logs
Soured Yogurt		1-5 Logs	4 Logs
Fresh Soft-Cheese		3-5 Logs	1-3 Logs
Brined Soft-Cheese		2-4 Logs	0-2 Logs
Butter		3-4 Logs	4-5 Logs
Cream		1-2 Logs	4-6 Logs

Table 7: Efficacy of hurdling with LTLT pasteurization posted by alcoholic peppermint extract on *C. albicans* clones.

Code book	Macfurland Experimental Log	Residual Fold Reduction Logs (CFU.ml^{-1})	
		After One Houre at Lab Temperature	After One Day at 4°C inside a refrigerator
YFFT	10^6 CFU.ml^{-1} or 6 logs Standardized	2	4
YYY		4	2
CBGG		Zero	Zero
YST		3	1
MM		5	4
MMM		6	5
CRS		4	2
BUS		4	2
CFT		2	1
YYG		4	1

Table 9: Efficacy of hurdling with LTLT pasteurization posted by watery peppermint extract on *C. albicans* clones.

Code book	Macfurland Experimental Log	Residual Fold Reduction Logs (CFU.ml^{-1})	
		After One Houre at Lab Temperature	After One Day at 4°C inside a refrigerator
YFFT	10^6 CFU.ml^{-1} or 6 logs Standardized	2	2
YYY		2	Zero
CBGG		Zero	Zero
YST		1	Zero
MM		3	1
MMM		4	2
CRS		2	1
BUS		2	1
CFT		1	Zero
YYG		2	1

resist thermal shock; or the reason may be due to the fact that the isolates are smooth when conducting the experiment, *i.e.* they possess the active entero-haemolysins and the surface protein catalyzed to manufacture and secretion (P60) in which, haemolysins weakened thermal tolerance of recovered clones. It was found that Rough isolates do not have a complete, effective and active haemolysins due to non-synthesis of P60, which will in some way lead to non-separation or incomplete-dissociation cells were transformed from spherical cocci with a balanced cell envelope into long chains like sausage-chain with a change in the chemical structure of their cell wall (becoming thick). This will stimulate the manufacture and secretion of thermal shock proteins (HSPs) with the increased elasticity of its cellular wall to withstand heat for a time; or the reason may be due recovering from clinical condition, i.e. exposure to sub lethally temperatures during the development of fever in infected cases and hence the ability to manufacture and secrete HSPs and consequently their sudden tolerance due to accumulative adaptation of their sensory ecosystems thermally in the normal state. However, Jay (2005) has another complementary explanation for the above explanations based on the fact that the resistance of microbes, especially germs to heat (such as pasteurization), depends on the water content (moisture level) of the food contaminated with the bacterium, where milk is about 75% of it is water and this will result in the start of the thermal process (such as pasteurization) to increase the release of active groups (milk groups) from milk proteins and bodies of *C. albicans*, especially free sulfur groups (free SH-groups), which works to increase the bounding of milk water with *C. albicans* proteins and then break down (Denaturation) in the presence of high temperature generate for a certain period of time due to the increase of degradation of abnormal *C. albicans* proteins in these ranges, while we need more heat and longer duration in the case of *C. albicans* protein in food with a lower moisture level (water content) of milk such as cheese or cream. Antimicrobial activity against foodborne pathogenic and spoilage microorganisms has been reported for these crude active ingredients of peppermint (*Mentha piperita*). These extracts significantly decreased the morphological changes of *C. albicans*, reduced biofilm formation, and disrupted their mature biofilms as well as decreasing the production of virulence-associated exoproteins by *S. aureus* at sub-inhibitory concentrations in a dose-dependent manner (Li *et al*., 2011). Exposure of *C. albicans* to essential oils and its vapor downregulated the expression of various genes, such as secreted aspartyl proteinases and hyphal wall protein 1 (HWP1). Indeed, various monoterpenes (citronellal, menthol and carvacrol)

Table 10: Efficacy of hurdling with HTST pasteurization posted by watery peppermint extract on *C. albicans* clones.

Code book	Macfurland Experi-mental Log	Residual Fold Reduction Logs ($CFU.ml^{-1}$)	
		After One Houre at Lab Temperature	After One Day at 4°C inside a refrigerator
YFFT	10^6 $CFU.ml^{-1}$	1	Zero
YYY	or 6 logs	1	Zero
CBGG	Standardized	Zero	Zero
YST		Zero	Zero
MM		1	Zero
MMM		2	Zero
CRS		Zero	Zero
BUS		Zero	Zero
CFT		Zero	Zero
YYG		Zero	Zero

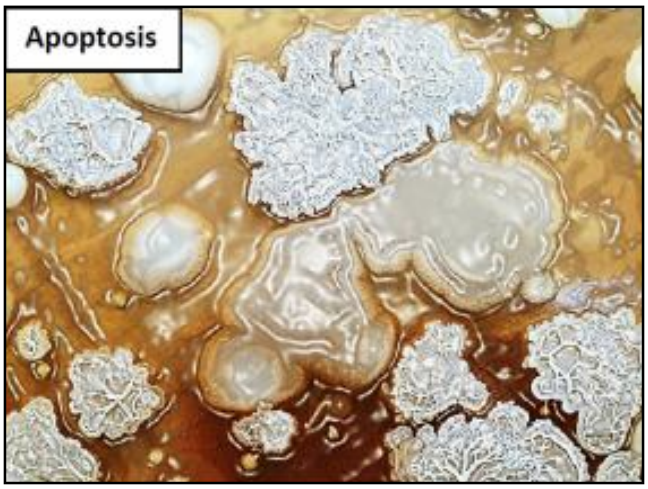

Fig. 2: Efficacy of Hurdle processing with thermal LTLT and HTST Pasteurization plus alcoholic and watery extracts of peppermint on *C. albicans* clones. Noticed residual dead bodies of fungi as apoptosis after dual processing regimes.

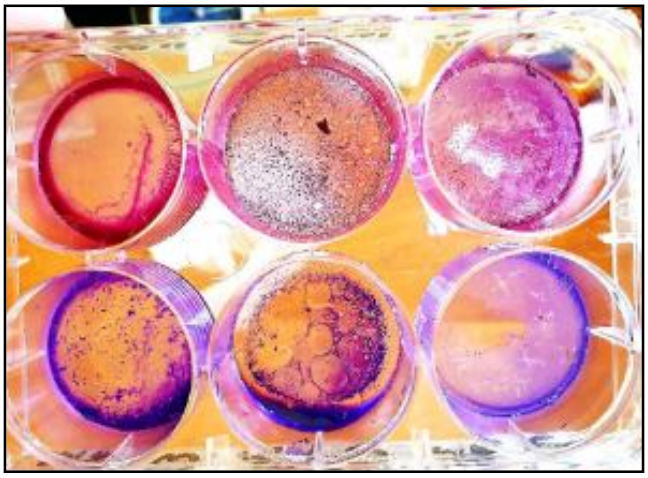

Fig. 3: Biofilm inhibition assay of *C. albicans* processed with peppermint extracts. From point of view cascaded right control, middle treated with alcoholic extract and left treated with watery extract.

of peppermint causing disruption in cell membrane & biofilm of *C. albicans* ending with apoptosis & death (Benzaid *et al*., 2019). Combined hurdle processing regimes with thermal processing cascaded by peppermint were efficient for reducing and combating the denominator to the limits that not harmful while pasteurization regimes alone could reduce majority but not thermoduric strains.

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