Antimicrobial activity of Curcumin upon pathogenic microorganisms during manufacture and storage of a novel style cheese 'Karishcum".

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Abstract: A survey study was made to evaluate the level of microbial contamination of 35 samples of Karish cheese retailed in Cairo area in compare with the Egyptian standard (ES-1008-2000). Survey results indicated that the brand cheese showed the highest quality, while the street & shop made and vend cheese samples showed the higher the ACC and the worst mycological quality, as reached $>10^3$ cfu/g for each of mold and yeast counts. So, 57% of Karish cheese samples would not be accepted due to the high mycological counts. Coliform group and *Escherichia coli* as fecal indicator contamination were detected in 57 and 25.7 % of the retailed Karish cheese samples, in averages of $\sim10^3$ cfu/g of the Coliform counts, respectively. However, *Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes* were isolated from 17, 8.5 and 2.8 % of the total Kariesh samples, respectively, but any was isolated from the brand cheese. A novel style of Karish cheese was made by adding *Curcuma. Longa* (Curcumin or Turmeric) at a rate of 0.3% (w/v), to obtain a new dairy product "Karishcum". A primary experiment was done to determine the correct percentage of Curcumin addition to cheese milk to get good taste and long shelf-life. A panel test was carried out to determined organoleptic properties of "Karishcum". The behavior of pathogenic bacteria in artificially contaminated during cold storage period at 7°C+1,for 14days, revealed that addition of aqueous Curcumin extract (0.3%) achived a reduction of bacterial counts obout one log of *Salmonella typhimrium*, tow log of *Pseudomonas aurogenosa* and *E.coli0157:H7*, respectively. Meanwhile each *of S.aureus, B.cereus* and *L.monocytogenes* were vanished at the end of the cold storage period (14 days).
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1. Introduction

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants. *Curcuma longa* is a medicinal plant that botanically is related to *Zigberiaceas* family, (Chattopadhyay et al, 2004). Turmeric powder, derived from the rhizome of *Curcuma. longa*, is commonly used as a spice, food preservative, and food coloring agent, (Aggarwall et al,2007.,Di Mario et al 2007.,Menon and Sudheer,2007).It also has a long history of therapeutic use ,(chattopadhyay et al ,2004). Curcumin is an oil soluble pigment, (Stan kovic, 2004), according to (Kurien et al., 2007), Curcumin has extremely limited water solubility, and however the water solubility of Curcumin could be increased from 0.6 ug/ml, to 7.4 ug/ml, (12 fold increase) by the use of heat. It is also soluble in ethanol and acetone,(Joe et al, 2004). The chemical structure of Curcumin is well known. Curcumin have a wide spectrum of biological actions such as anti-inflammatory, (Puntihavathi et al.,2000) ,Siddiqui et al., 2006), antioxidant , (Mohammadi et al., 2005).,Menon and sudheer 2007).), anticancer.,(Lotempio et al .,2005) ,antidiabetic .,(Aggarwal et al .,2007)), antiallergic, (Suzuki et al.,2005),antiviral , (Si et al.,2007), antiprotozoal,(Reddy et al., 2005), and antifungal activities ,(Chatopadhyay et al .,2005).It contains a mixture of powerful antioxidant phytonutrients known as Curcuminoids and inhibits cancer at initiation, promotion and progression stages of tumor development. It is a strong anti-oxidant which supports colon health, exerts neuroprotective activity and helps to maintain a healthy cardiovascular system, (Luthra et al., 2001).

A recent trend in cheese manufacture is production of nature flavored cheese made in short time with highly nutritive value and good microbiological quality as for human consumption, (Abou-Zeid., 1992, Hussein., 2004 and Foad et al., 2006). Therefore, the present study was conducted to use *Curcuma. Longa* in the preparation of new style Karish cheese named (Karishcum), based on traditional Egyptian Karish cheese. In Karishcum, the shelf life potential, panel test, pH values and growth & survival of some food borne pathogens is a target of this study either in fresh cheese or during storage especially after the survey was done. This study also supports the increasing interest in the utilization of medicinal plants for production of novel styles of food products for the beneficial of consumer's health and wealth.

2. Materials and methods

Material :

1-Samples:

35 of Karish samples were collected from great Cairo governorate, in sterilized plastics pages, and kept in refrigerator for microbiological analysis .

1.Pathogenic bacterial strains source :

Escherichia coli 0157:H7, Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus and *Salmonella typhimurium as reference* and test strains were obtained from the Central Public Health Laboratories; (Ministry of Health).These strains were maintained and propagated in tryptone soya broth and agar until use.

2- Curcumin powder: Curcumin was purchased from retail spice-Cairo market.

3-Milk: Fresh buffalo' s skim milk was obtained from the herd of faculty of Agriculture, Cairo University.

Methods:

1-Culture preparation (test strains):

The test pathogenic strains were routinely transferred into brain heart infusion broth (oxoid), incubated at 37°C for 24 hr. After sufficient growth, turbidity was observed; the cultures were diluted in saline solutions to an appropriate inoculum size, by diluting the strain suspension equivalent to the turbidity standard No.1 of the McFarland nephelometer scale (McFarland, 1907).

2-The diluted Curcumin: The solution was prepared according to Murad (1998).Aqueous extract prepared from Curcumin powder by boiling of 10% (w/v) . Then Left for sedimentation.

3-Antimicrobial activity of Curcuma Longa in nutrient agar medium: Was done according to Barry (1986).Using an agar diffusion method (disk assay) used as a qualitative method.

4-Karishcum cheese making:

Karish cheese was prepared according to Fahmi (1960) and modified with addition of Curcuma Longa powder as follows: The bulk milk was divided into three parts:

Cheese analysis:

Cheese samples were taken aseptically freshly and after 2, 3, 5, 7, and 14 days of storage periods. Samples were evaluated for their organoleptical properties, pH values were measured as well as microbiological properties.

Organoleptic properties:

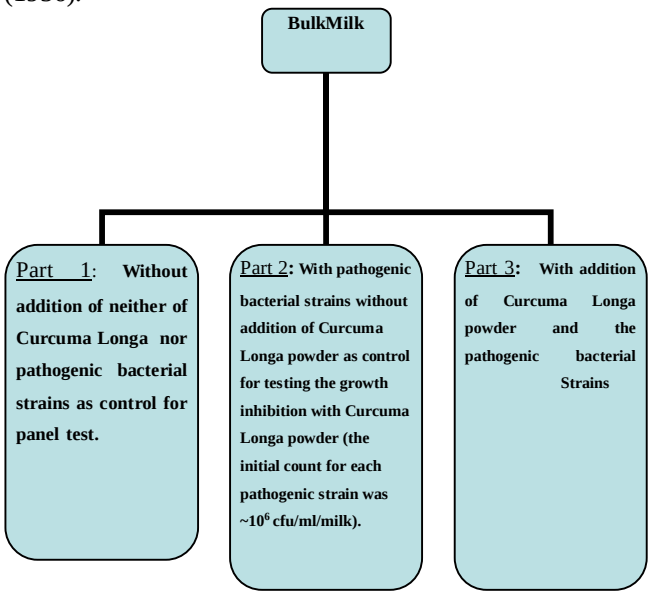
Organoleptic properties of cheese samples were evaluated according to EL-Koussy,(1966) when fresh ,48hr ,72hr, 5,7,14 days of refrigerated storage by ten expert panelists of members of Dairy Science and Technology, Department ,National Research Center.

pH values:

pH values was measured by direct inserting the combined electrode(digital pH model Hanna, 4817) into well-mixed samples.

Microbiological analysis:

Cheese samples (25/g) was homogenized for 1 /min with 225/ml of sterile solution (2% w/v) of sodium citrate. Methods of microbiological analysis were followed according to, APHA (1992) for: Total bacterial count on plate count agar (Oxoid),total coliform on Violet Red Bile agar (Oxoid),Mould and yeast on acidified Potato Dextrose agar(Oxoid),*Staphylococcus aureus* on Baird Parker medium (Oxoid),*E.Coli o157:H7* was determined on Sorbitol MaConkey agar(Oxoid), *Bacillus cerues* was enumerated on Manitol Egg Yolk Polymyxin agar (Oxoid) following the surface plate method and incubated at 37°C for 18-24hr ,Holbrook&Andreson, (1980),*Salmonella Typhimurium* was enumerated by surface plating technique on Salmonella Sgidella agar (Ox0id),*Listeria monocytogenes* was enumerated on Oxford Selective agar base (Oxoid) supplemented with listeria selective supplement using surface plating technique, plates were incubated at 32°C for 48hr,Curtis et al (1989), and *Lactic Acid Bacteria* (LAB) on Elliker Medium according to ,Elliker et al (1956).



3. Results and Discussion

Microbiological quality of Karish cheese in Cairo governorate:

Thirty five samples were collected from great Cairo governor as follows (15 street vended, 10 milk shops and 10 branded samples). Results as shown in Table (1) for aerobic colony bacterial counts (ACC) and molds and yeasts counts (M/YC) revealed that there were wide differences between the cheese's sources, since

the brand cheese showed the highest quality. While the street and shop made and vend cheese samples showed the higher the ACC and the worst mycological quality, as reached $>10^3$ cfu/g for each of mold and yeast counts. So, 57% of Karish cheese samples would not be accepted due to the high mycological counts, exceeded 10 cfu/g mold or 400 cfu/g yeast, according to the Egyptian Standard ES 1008-2000, as shown in Table (4)

In this respect, similar results were obtained for ACC and MYC by Abou Dawood et al., (2005), but higher than that found by El Ghaish (2004) and Tawfek et al., (1988).Coliform group and *Escherichia coli* as fecal indicator contamination were detected in 57 and 25.7 % of the retailed Karish cheese samples, in averages of $\sim10^3$ cfu/g of the Coliform counts, respectively as shown in Table (2) .Also, *Salmonella spp* , was only detected in 6.6 % of the street vend. According to the Egyptian Standard (ES 1008-2000) ,there were 57 and 25.7 % of the samples would not accepted due to the high counts of Coliform and the presence of *E.coli* , respectively ,as shown in Table (4).

In this respect ,similar results were obtained by(Abou Dawood et al, 2005) for the Coliform counts in Karish samples higher than the Egyptian standard ,but these counts were less than that reported by Moussa et al.(1984) and (Kaldes, 1997) and higher than those found by (Ahmed ,1988) and (El Ghaish ,2004).controversially , *Salmonella spp.* was detected in 2.7 of the samples contradict the results obtained by (Tawfek et al.,1988) and (El Ghaish ,2004) who did not found these bacteria in any of Karish cheese samples, and they attributed that to the low pH values of Karish cheese.

However, *Staphylococcus aureus , Bacillus cereus and Listeria monocytogenes* were isolated from 17, 8.5 and 2.8 % of the total Kariesh samples, respectively, but any was isolated from the brand cheese as shown in Tables (3) .Hence, street and shop made/ vend cheese samples would pay much attention to that variety in particular from the side of hygienic quality . Furthermore and legally, the presence of these pathogens in 17 % of the total Kariesh samples make them microbiologically not accepted according to the ES 1008-2000 ,(as shown in Table ,4). Results obtained by Abou Dawood et al.(2005) reveal much higher incidence and counts for *S. aureus* than that found in the current study, as they found this bacterium in all of the Kariesh samples .These counts were less than those found by Naguib et al.(1986) and Kaldes (1997) but higher than those reported by El Ghaish (2004). Generally, this indicate how is the inferior quality and hazardous food as Karish cheese as an etiology for foodborne illness for Cairo locals. So there is a great need for rising up, developing and spreading the hygienic knowledge, attention and control measures where Karish cheese is made, handled and served for the public health good and particularly when exportation is attempted.

Table (1): Microbiological quality of Karish cheese samples collected from central Cairo area.

	Aerobic colony Count Cfu/g			Mould count Cfu/g			Yeast count Cfu/g		
	min	max	avrg	min	max	avrg	min	max	avrg
15(street vend)	$5x10^6$	$2x10^9$	$4x10^8$	10^3	$5x10^7$	$5x10^4$	10^3	10^6	$2x10^5$
10 (milk shops)	10^6	$7x10^8$	$5x10^7$	10^3	10^5	10^4	10^5	10^6	$3x10^4$
10 (branded)	10^6	$5x10^7$	10^7	0	10^2	2x10	0	3x10	3x10
Total 35 samples	10^6	$2x10^9$	$4x10^7$	0	$5x10^7$	$5x10^3$	0	10^6	$5x10^3$

Table (2): The Gram negative indicator and pathogenic bacteria of Karish cheese samples collected from central Cairo area.

No of samples/ Source	Coliform Cfu/g				*E. coli*	*Salmonella*
	min	max	avrg	%	%	%
15(street vend)	0	10^7	10^4	73	40	6.6
10 (milk shops)	0	10^6	10^3	60	30	0
10(branded)	0	3x10	3x10	20	0	0
Total 35 samples	0	10^7	10^3	57	25.7	2.8

Table (3): The Gram positive indicator for pathogenic bacteria in Karish cheese samples collected from central Cairo area.

No of samples/ Source	*S.aureus* Cfu/g				*B. cereus* Cfu/g				*L.monocyt.*
	min	**max**	**avrg**	**%**	**min**	**max**	**avrg**	**%**	**%**
15(street vend)	**0**	**$3x10^3$**	**5x10**	**27**	**0**	**10^2**	**3x10**	**20**	**6.6**
10 (milk shops)	**0**	**10^2**	**3x10**	**20**	**0**	**0**	**0**	**0**	**0**
10 (branded)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total 35 samples	**0**	**$3x10^3$**	**3x10**	**17**	**0**	**10^2**	**3x10**	**8.5**	**2.8**

Table (4): the microbiological quality of the central Cairo retailed Karish cheese due to the specification of the Egyptian Standard* ES-1008-2000

No of samples/ source	**% fit in& out(parameters) to ES 1008/2000**						**% total**	
	Mold %	**Yeast %**	**Coliform %**	***E.coli* %**	***L.monocyt.* %**	**Other Pathogenic Bacteria %**	**Fit in**	**Fit out**
15(street)	**53.3**	**86.6**	**73**	**40**	**6.6**	**27**	**13.4**	**86.6**
10(shops)	**50**	**70**	**60**	**30**	**0**	**20**	**30**	**70**
10(brand)	**0**	**0**	**20**	**0**	**0**	**0**	**80**	**20**
Total 35	**37**	**57**	**57**	**25.7**	**2.8**	**17**	**37**	**63**

* ES 1008/2000, cheese sample should be free from *E. coli*, *Listeria monocytogenes* and other pathogens and counts should not exceed 10 cfu/g for each of molds and Coliform and 400 cfu/g for yeast

Table (5): Water Curcumin concentrations effect on different pathogenic bacteria

Curcumin concentrations / test bacteria	**Inhibition zones in mm***			
Water boiled Curcumin concentrations w/v	**0.2%**	**0.3%**	**0.6%**	**0.8%**
S. aureus	**6**	**7**	**9**	**9**
E. coli	**6**	**7**	**9**	**9**
B.cereus	-	-	**7**	**8**
Salmonella	**6**	**6**	**7**	**8**
Pseudomonas	**6**	**6**	**9**	**9**
Listeria monocytogens	-	-	-	-
Water not heated Curcumin concentrations w/v	**0.2%**	**0.4%**	**0.6%**	**0.8%**
S. aureus	-	-	**6**	**6**
E. coli	-	**6**	**6**	**6**
B.cereus	-	**6**	**6**	**7**
Salmonella	-	**6**	**6**	**6**
Pseudomonas	**6**	**6**	**7**	**8**
Listeria monocytogens	-	-	-	-

* Before test the well diameter 5mm, + =6, ++ = 7, +++ = 8, ++++ = 9 mm

The inhibition zones as, antibacterial activity, of Curcuma Longa at different concentrations (0.2, 0.3, 0.6 and 0.8 %) are presented in Table (5). Results reveal that curcuma Longa extracts, boiled and not heat treated water suspensions, showed a relatively antibacterial activity, inhibition zones, against some of the tested strains of pathogenic bacteria. The boiled water curcuma extracts showed the higher the activity than the not heated Curcumin extracts, 2 to 3 folds, though the obtained results were in consent with the findings by, Stankovic (2004).

The most susceptible bacteria were in order *pseudomonas*, *E. coli*, *Salmonella*, *S. aureus* and *B. cereus*. Results reveal also that the increase the Curcumin concentration levels, 0.2 to 0.8%, the wider the inhibition zones, 6 to 9 mm, and consequently this mean the higher the bacterial growth suppression.

Generally,from the obtained results Curcumin showed a wide spectra of antibacterial activity against Gram negative and positive bacteria and were in concomitant with those reported by, Tajbakhsh et al (2008), who reported that Curcumin was effective against *S. aureus and Pseudomonas aeruginosa.* Also, the great antibacterial activity of Curcuma Longa against *Bacillus spp., B. subtitles* in particular was reported by Naz et al (2010).

Organolyptic evaluation:

Results obtained by the panelists revealed that Karish cheese with Curcumin (Karishcum) gave the highest score comparing with panel Karish cheese (control) over all storage period, table (6). During cold storage period the flavor and taste of Karishcum cheese samples were not markedly changed, while the flavored and taste of the control cheese samples was slightly got changed after 14 days ,(sour taste& flavor). Therefore, it could be concluded that preparing Karish cheese with Curcumin enhanced its organoleptic properties, flavor and taste in particular and announced an appreciation by the panelists. Also, Karishcum showed better keeping quality and longer shelf-life more than plane Karish cheese (control).Abou-Zeid (1992) reported similar findings for the enhancement of organoleptic properties of Domiati cheese containing Parsley or rocket. Also the current results are similar to those obtained by Murad, (1998) who used some natural essential oils, and successfully applied these natural products to get better sensory characteristics and to elongate shelf-life of yoghurt.

Total Bacterial count, Lactic acid Count, Coliform counts, Mould &yeast count and PH values of "Karishcum" cheese during manufacture and cold storage period at 7±1°C. :

Data presented in Table (7) showed that, total bacterial count were ranged from 6.93/Log Bacterial Count when fresh to 2.3/LBC at the end of storage period for control cheese. Meanwhile in the fresh cheese treated with Curcumin gave a count of 5.4/ LBC and not detected at the end of storage period Table (7). Mould & yeast, were not detected in both cheese samples wither made with Curcuma Longa until the end of cold storage period or without it until 7 days of cold storage at level of 2.3/LBC, and reached to 3.6/LBC, after 10 days cold storage period and undetectable at the end of the storage period. Coliform bacteria count was not detected for both cheese samples when fresh and at the end of cold storage period, Table (7).

These results may be due to the pasteurization of milk used for manufacture of both cheese samples, and could be also due to the lower levels of pH values measured during storage of cheese. Lactic acid bacteria count, table (7), was within normal counts when fresh and during storage period for both cheese samples and slightly lower in cheese made with Curcumin at the end of storage period. These results may be attributed to the antimicrobial activity of Curcumin against the test strains of pathogenic and non pathogenic bacteria. The results of pH values were the same for both cheese samples when fresh or at the end of cold storage period, table (7). These results are in agreement with those obtained with Murad (1998), El-Kholy(2007),Tajbakash etal(2008),Sara Burt(2004) and Shagufta etal(2010),who found the same findings during their researchers about pathogenic and non pathogenic bacteria during their studies on soft cheese.

Table (6) Average of organoleptic properties of Karish cheese made with Curcumin (Karishcum) during cold storage at 7±1°C for 14 days.

Treatment	Storage period/days	Body& Texture	Color& Appearance	Flavor	Total
C	Fresh	17.7	24.9	44.5	87.1
K		18.0	26.0	46.0	90.0
C	7 Days	15.3	21.9	41.4	78.6
K		17.9	26.5	45.9	90.3
C	14 Days	15.0	20.0	41.5	76.5
K		17.5	26.1	44.5	88.1

C= Control Karish cheese. K=Karish cheese made with Curcumin.

Table(7) total Bacterial count, Lactic acid Count,Coliform counts,Mould&yeast count and PH values of "Karishcum"cheese during manufacture and cold storage period at 7±1°C.

Storage period/days	*Total Bacterial count*		*Lactic acid bacteria*		*Cliform group*		*Mould&Yeast*		*PH values*	
	C	T	C	T	C	T	C	T	C	T
0	6.93	5. 40	6.60	7.00	ND	ND	-ve	-ve	4.60	4.40
3	6.80	5.70	7.25	7.50	ND	ND	-ve	-ve	4.40	4.41
5	5.69	5.20	6.84	7.60	ND	ND	-ve	-ve	4.00	3.80
7	5.30	3.80	5.20	6.10	ND	ND	2.30	-ve	3.80	3.80
10	5.21	3.20	4.90	4.70	ND	ND	3.60	-ve	3.80	3.80
14	2.30	ND	4.70	4.50	ND	ND	-ve	-ve	3.60	3.60

C=control T=cheese treatment with Curcuma Longa -ve=negativeAll numbers= are Log counts ND=not detected

Antibacterial activity of Curcuma Longa (Turmeric) In Karish cheese during manufacture and cold storage period:

The behavior of pathogenic bacteria in artificially contaminated Karish cheese made with added of Curcumin(Karishcum) during cold storage period at 7°C+1 for 14 days, individually, are shown in table (8). The obtained results revealed that addition of aqueous Curcumin extract (0.3%) achived a reduction of bacterial counts obout one log of *Salmonella typhimrium.* two log of *Pesudomonas aurogenosa and E.coli0157:H7,* respectively.Meanwhile each of ,S.aureus, B.cereus and L. monocytogenes were vanished at the end of cold storage period (14 days). This in respect with control cheese during the same period of cold storage period. The current results were in concomation with those obtained by Murad,(1998), reported that some natural aqueous extracts had antimicrobial activity upon wide variety of yeasts (*Kiuyveromyces morxianus, klu.latis, Candida lipotica, C.utilis, Hanenula anomola,Asperagillus niger, Asp. Ochroceus* and *Asp fumigates),* and bacteria (*Bacillus polymexa , B. thuringinesis* and *B. subtilis*).Ahmed and Ebraheim (2010) designed a study to evaluate the addition of some edible plants including Cayenne, green pepper, parsley and dill to Karish cheese. Cayenne and green pepper extracts showed highest activity followed by dill and parsley against *S.aureus*. Addition of Cayenne or green pepper to Karish cheese during manufacture reveled that both plants were able to reduce *S. aureus* population to undetectable level within the first and second days of storage. In addition, Sara Burt (2004) she study the antibacterial activity of essential oils (EOs) against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Shigella dysenteria*, *Bacillus cereus* and *Staphylococcus aureus* at levels between 0.2 and 10 µl ml^{-1}. Gram-negative organisms are slightly less susceptible than gram-positive bacteria. A number of EO components has been identified as effective antibacterials, e.g. carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde and cinnamic acid, having minimum inhibitory concentrations (MICs) of 0.05–5 µl ml^{-1} in vitro. A higher concentration is needed to achieve the same effect in foods. Studies with fresh meat, meat products, fish, milk, dairy products, vegetables, fruit and cooked rice have shown that the concentration needed to achieve a significant antibacterial effect is around 0.5–20 µl g^{-1} in foods.

Table (8). Viability of pathogenic bacteria in Karishcum cheese during cold storage period

Storage-period/days	*Ps. arugenosa*		*E.coli O157:H7*		*S.aureus*		*B.cereus*		*Sal. typhimrium*		*L. monocytogenes*	
	C	T	C	T	C	T	C	T	C	T	C	T
0	5.82	5.21	5.15	4.99	5.77	4.60	5.64	5.00	5.21	3.39	6.00	5.10
3	4.93	4.10	4.81	4.99	4.47	4.00	4.90	3.50	4.51	3.00	4.95	4.10
5	4.48	3.78	4.34	4.00	3.40	2.60	4.35	3.30	4.31	3.70	4.50	3.20
7	4.20	3.10	4.00	3.10	2.40	2.00	4.00	3.17	4.10	3.40	3.80	2.50
10	3.50	2.60	3.23	2.85	2.15	1.50	3.31	2.90	3.28	2.70	3.50	-ve
14	3.28	1.21	3.10	1.00	2.15	-ve	3.00	-ve	3.15	2.10	2.50	-ve

C=control Cheese treatment with Curcuma Longa - ve=negative All numbers= are Log counts

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