Accelerated Ripening of UF Feta-Cheese by Using Freeze-Shocked Culture of *Lactobacillus helveticus* or Barley Extract

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ABSTRACT

The effect of using freeze-dried shocked culture or barley extract in the manufacture of UF Feta-cheese made from ultrafiltreted retentate containing different levels of either barley extract at ratio of 0.05% and 0.1% respectively, or freeze-shocked culture of *L. delhrueckii subsp. helveticus* at ratio of 1% and 2% respectively, on ripening process and quality was studied. Addition of barley-extract did not significantly affect the moisture, fat, total nitrogen or salt content of the resultant cheeses, but significantly increased its acidity, while the incorporation of the freeze-shocked starter cultures had no significant effects on the gross chemical composition of cheeses. These additives significantly increased the formation of soluble nitrogenous compound, free volatile fatty acids, flavour intensity and improved the body and texture characteristics of UF Feta cheese. Especially the cheese made with 0.1% barley extract and 0.2% of freeze-shocked starter culture of *L. delhrueckii subsp. helveticus* which resulted in higher quality after 15 - 30 days of ripening than the other treatments. Microbial counts during preservation of both types of cheese increased steadily and reached similar levels, with the exception of Coliform-bacteria. All types of cheese produced with barley extract or freeze-shocked culture as a starter were approved and accepted by the panel during the preliminary sensory evaluation compared to commercial feta- cheese.

KEY WORDS: UF Feta-cheese; ripening acceleration; barley extract; *Lactobacillus helveticus* and cheese quality.

INTRODUCTION

Ripening of cheese involves microbiological and biochemical changes resulting in its characteristic flavour and texture of different cheese variaties. Proteolysis is the most important and complex primary biochemical events that occur in most cheeses during ripening (McSweeney, 2004). Different methods developed for accelerated cheese ripening have been reviewed by several investigators (El-Soda 1993 and Wilkinson & Kilcowley, 2005). One of the possible approaches is to enhance the enzyme systems involved in the breakdown of casein into different soluble peptides and amino acids which serve as cheese flavour precursors. Increasing the level of starter proteolytic enzymes in cheese by using the physically modified starters (heat or freeze-shocked) have been attempted to accelerate the ripening of many cheese varieties (Johnson *et al*., 1995; Kebary *et al*., 1996, 1999 and Madkor *et al*., 2000), who could enhance cheese flavour without development of bitter taste. Barley extract appears to be a potential source of dipeptidase, proteinase and carboxy-peptidase that can be used in cheese making and to the accelerate ripening of Cheddar, Gouda and Edam cheeses (Frey, 1986; and Zaki and Salem 1992). In the present study, attention was focused on evaluating the effectiveness of adding freeze-shocked starter cultures of *L. delbrueckii subsp. helveticus* and barley extract on the quality and ripening properties of UF Feta-cheese made from ultrafiltreted retentate.

MATERIALS AND METHODS

Materials

Milk Samples

Fresh cow's milk (3% fat and 8.50% solids not fat) and Ultrafiltreted milk retentate (35% total solids) of the same milk were obtained from the International Dairy and Foods Company (Green Land, Al-Asher men Ramadan City, Egypt).

Rennet and microbial strains

A standard animal rennet powder (1:100000) was obtained from L.C.Glad Company A / S, Copenhagen, Denmark.

Lactic starter cultures composed of *L. delbrueckii subsp. bulgaricus*, *S. salivarius subsp. thermophilus* and *L. delbrueckii subsp. helveticus* were obtained from Chr- Hansen's Company (Horsholm, Denmark).

Other materials

Glucono-Delta-Lactone (GDL) was obtained from the Sigma Chemical Company, (St. Louis, Mo. USA).

Barley was obtained from El-Ahram Beverages Company, El-Azazi, Sharkia Governorate, Egypt and used in the preparation of enzymes.

Methods

Preparation of freeze-shocked culture

Starter culture of *L. delbrueckii subsp. helveticus* was freeze-shocked as described by Frey *et al*., (1986), as follows: the strain of *L. delbrueckii subsp. helveticus* was grown to three successive subcultures in

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reconstituted skim milk (11.5%TS) for 16-18 hrs at 37°C. From the third subculture, the inoculum for cheese making was prepared by growing the cells in MRS broth (DeMan *et al*., 1960) for 10-12 hours at the same temperature previously mentioned and a constant pH of 5.8 (A 50% NH_4OH, solution was used to neutralize acid production). Then, the cultures were harvested by centrifugation at 10000 xg at 4°C, and washed three times with 0.01 N phosphate buffers, pH 6.5 and suspended in this buffer. The cells suspensions were frozen at -20°C for 24 hours. Freeze-shocked cultures was thawed before being used

Barley extract preparation

Barley extract was prepared according to the procedure given by Frey *et al*., (1986). Dry barley grains were soaked in tap water for 24 hrs, then removed, and germinated for about 4-5 days; during this, the grains were sprayed with water. One kilogram of germinated barley was blended using and mixed with 2 liters of 0.01 N phosphate buffer, pH 4.5, a blender was incubated for one hour at 5°C. The homogenate mixture was centrifuged at 5000xg for 30 min. Ammonium sulfate was added to the supernatant at the ratio of 40% (w/w), incubated for two hours at 5°C and centrifuged at 5000 xg for 30min. The supernatant was discarded and the precipitate was added to UF Feta-cheese at the ratios of 0.05 and 0.1%.

Cheese manufacture

UF Feta-cheese was made according to the procedure described by Tamime and Kirkegaard (1991), with some modifications. Cow's milk retentate (35% total solids) was heated to 75°C / 15 sec., and then cooled to 35°C. The retentate was mixed with 0.5% starter culture containing *L. delbrueckii subsp. bulgaricus* and *S. salivarius subsp. thermophilus* at a ratio (1:1), and 0.02% calcium chloride. GDL was used in an amount that decreased the pH of retentate to be around 5.2, and then the mixture was salted (4%) and divided into five portions and treated as follows: a) the first and second portions were incubated with 1% and 2% of freeze-shocked starter culture of *L. delbrueckii subsp. helveticus*, respectively, then renneted and converted into UF Feta-cheese. b) The third and fourth portions, were treated with 0.05% and 0.1% of barley extract, respectively, then renneted and converted into UF Feta-cheese. c) The fifth portion was renneted without additives and converted into UF Feta-cheese as a control. The resultant cheeses of all treatments were pickled in 12% brine solution and stored at 8 ± 2°C for 8 weeks. Three replicates were made for each treatment. Samples were taken when fresh and after 2, 4 and 8 weeks of cheese ripening period for chemical analysis, bacteriological examination and organoleptic evaluation.

Chemical analysis

All cheese samples were chemically analysed for moisture and salt contents by the method of IDF (1982); titratable acidity, fat content and total volatile fatty acids were measured according to standard methods (AOAC, 2000). Total nitrogen (TN), and non-protein nitrogen (NPN) contents were determined by the semi-micro Kjeldahl method described by IDF (1993), the water soluble nitrogen (WSN) content in cheese was determined according to Kuchroo and Fox (1982). And phospho-tungstic acid 5% soluble nitrogen (PTA-SN or AN) was determined according to Jarrett *et al.,* (1982). Total volatile fatty acids in cheese samples were determined according to the method of Kosikowski (1982).

Microbial examination

Ten g of cheese sample were placed in sterile mortar and homogenized with 90ml of sterile ringer solution. Total bacterial counts of UF Feta-cheese was determined according to the American Public Health Association, (APHA,1992) by plating suitable dilutions in duplicates on nutrient agar medium (Oxoid limited, 2005) with incubation at 37°C for 3 days. Yeasts and moulds were cultivated on Potato Dextrose Agar (Merk, Germany) (pH3.5) at 24°C for 4 days. Coliform bacteria were determined on MacConkey Agar (Merk, Germany) at 37°C for 24 hours.

Organoleptic evaluation

Organoleptic properties of UF Feta-cheese were assessed by nine staff members of Food Science Department, Faculty of Agriculture, Zagazig University. Cheeses were evaluated for sensory attributes according to Vafopoulou *et al.,* (1989) with maximum score points of 40 for flavour and 20 for body and texture.

Statistical analysis

Experiments were performed in triplicate and ranked order of the means were analysed using one way analysis of variance, ANOVA (on the basis of complete randomized design) from COSTAT software (Version 3.6).

RESULTS AND DISCUSSION

Gross Chemical Composition

Table (1) shows that the addition of freeze-shocked culture of *L. delbrueckii subsp. helveticus* at levels of 1% and 2% did not significantly affect the composition of the resultant cheese. Several investigators showed that using of freeze-shocked cultures did not affect the composition of Gouda cheese (Kim *et al.,* 1994), Bulgarian white brined cheese (El-Neshawy and

Table 1. Gross chemical composition of UF Feta - cheese as affected by varies levels of freeze-shocked starter culture of *L. helveticus* and barley extract

Ripening period (weeks)	Feta-cheese containing					LSD
	Control	Barley Extract		Freeze-shocked culture *L. helveticus*		
		0.05 %	0.1 %	1 %	2.%	
			Moisture %			
Fresh	60.67[a]	61.08[a]	61.92[a]	60.86[a]	60.74[a]	4.21
2	58.48[a]	59.38[a]	59.66[a]	58.02[a]	58.18[a]	2.57
4	57.56[a]	58.49[a]	58.87[a]	57.94[a]	57.89[a]	2.46
8	55.83[a]	56.72[a]	56.98[a]	55.0[a]	55.07[a]	2.7
			Fat /DM %			
Fresh	48.26[a]	48.76[a]	48.98[a]	48.38[a]	48.92[a]	2.29
2	49.59[a]	49.09[a]	49.67[a]	49.8[a]	49.74[a]	2.26
4	50.2[a]	50.86[a]	50.54[a]	50.97[a]	50.78[a]	3.28
8	51.19[a]	51.24[a]	51.08[a]	51.0[a]	51.09[a]	3.69
			Total N /DM%			
Fresh	6.0[a]	6.04[a]	6.06[a]	6.02[a]	6.08[a]	0.71
2	5.73[a]	5.71[a]	5.73[a]	5.78[a]	5.7[a]	0.83
4	5.6[a]	5.56[a]	5.58[a]	5.69[a]	5.62[a]	0.51
8	5.54[a]	5.49[a]	5.53[a]	5.6[a]	5.58[a]	0.66
			Salt %			
Fresh	3.67[a]	3.8[a]	3.69[a]	3.74[a]	3.64[a]	0.69
2	4.89[a]	4.98[a]	4.76[a]	4.92[a]	4.88[a]	1.12
4	4.96[a]	5.0[a]	4.87[a]	4.98[a]	5.0[a]	0.79
8	5.21[a]	5.24[a]	5.18[a]	5.29[a]	5.31	1.26
			Titratable acidity %			
Fresh	1.26[a]	1.32[a]	1.36[a]	1.28[a]	1.3[a]	0.34
2	1.44[b]	1.89[ab]	2.08[a]	1.49[b]	1.48[b]	0.36
4	1.56[ab]	1.96[ab]	2.27[a]	1.63[a]	1.67[a]	0.74
8	1.77[ab]	2.3[ab]	2.46[a]	1.81[a]	1.86[a]	0.9

Means with the same letter in the same row are not significantly different.
Significant at 0.05 level ($P \leq 0.05$).
Fat/DM; fat/ dry matter
Total N/DM; Total nitrogen / dry matter
Titratable acidity; expressed as g / lactic acid / 100g yoghurt.

Groiave, 1988) and Domiati cheese (Aly, 1996). Addition of barley extract did not significantly affect the moisture, fat, total nitrogen and salt contents of the resultant cheese, but increased significantly the acidity compared with control. This was more pronounced with the increase in the level of barley extract added to the cheese. Similar results were obtained by Frey *et al*., (1986) in Cheddar cheese and Zaki and Salem (1992) in Edam cheese.

Ripening indices

As shown in Table(2), the levels of water soluble nitrogen (WSN),non-protein nitrogen (NPN) and amino acid nitrogen (AAN) were found to increase significantly in UF Feta-cheese during ripening by the addition of both freeze-shocked *L. delbrueckii subsp. helveticus* (at levels of 1% and 2%) and barley extract (at levels of 0.05% and 0.1%). This was more pronounced in cheeses containing the higher levels of freeze-shocked cultures of barley extract. The higher level of WSN, NPN and AAN of the experimental cheeses could be explained on the basis that the freeze-shocked cells have been found to lyses in the cheese to a greater extent than the normal cells (Bartels *et al*., 1987). This resulted in the greater release of intracellular enzymes: amino peptidases, dipeptidases and other peptide hydrolyzing enzymes (El-Soda *el al.*, 1993) which accelerate the rate of proteolysis during cheese ripening. The proteolytic action of barley extract may be attributed to its richness in the dipeptidases, proteinase and carboxypeptidase enzymes (Frey, 1986 and Frey *et al*., 1986).

Table (2) also indicates that the incorporation of both freeze-shocked *L. delbrueckii subsp. helveticus* and barley extract significantly increased the levels of volatile fatty acids of the experimental cheeses during

Table 2. Ripening indices of UF Feta - cheese as affected by various levels of freeze- shocked culture of *L. helveticus* and barley extract

Ripening indices	Ripening period (weeks)	Feta - cheese containing					LSD
		Control	Barley Extract		Freeze-shocked *L. helveticus*		
			0.05%	0.1	1%	2%	
SN/TN %	Fresh	5.43^{c}	8.13^{b}	9.26^{ab}	9.0^{b}	10.18^{a}	1.17
	2	8.02^{c}	12.0^{b}	14.21^{a}	12.98^{ab}	15.0^{a}	2.16
	4	10.91^{c}	15.72^{b}	16.17^{ab}	16.64^{ab}	17.91^{a}	1.97
	8	13.58^{d}	20.25^{c}	22.98^{bc}	21.62^{ab}	24.64^{a}	2.67
NPN/TN %	Fresh	1.7^{d}	3.1^{bc}	3.26^{b}	2.98^{c}	3.82^{a}	0.544
	2	2.15^{c}	7.39^{b}	8.73^{a}	8.0^{ab}	9``.04^{a}	1.05
	4	3.29^{c}	8.97^{b}	10.12^{ab}	9.38^{b}	10.81^{a}	1.39
	8	4.46^{d}	11^{c}	12.68^{ab}	11.21^{bc}	12.96^{a}	1.61
AN/TN %	Fresh	0.8^{b}	1.68^{a}	1.74^{a}	1.82^{a}	2.05^{a}	0.584
	2	1.24^{b}	2.54^{a}	2.71^{a}	2.45^{ab}	2.87^{a}	0.869
	4	2.02^{c}	3.58^{b}	3.95^{ab}	3.95^{ab}	4.39^{a}	0.807
	8	2.68^{c}	4.06^{b}	4.38^{b}	4.21^{b}	5.0^{a}	0.699
TVFA	Fresh	3.9^{b}	4.3^{ab}	4.6^{a}	4.6^{a}	4.8^{a}	0.5824
	2	7.2^{d}	13.2^{c}	14.5^{ab}	14.0^{bc}	15.2^{a}	1.19
	4	8.7^{d}	20.9^{c}	23ab	22.8^{b}	24.6^{a}	1.7
	8	11.2^{d}	27.5^{c}	28.9^{bc}	28.2^{ab}	29.7^{a}	1.31

Means with the same letter in the same row are not significantly different.
Significant at 0.05 levels ($p \leq 0.05$).
TN; total nitrogen NPN; non-protein nitrogen
SN; soluble nitrogen AN; amino acids nitrogen
TVFA; total volatile fatty acids (ml N/10 NaOH/100g)

ripening. This was more significantly pronounced with the increase in the level of freeze-shocked *L. delbrueckii subsp. helveticus* or barley extract added to the cheese. The higher levels of the volatile fatty acids in the all treatments could be due to the higher levels of free amino acids which may serve as precursors for fatty acids (Nakae and Elliott, 1965). Several investigators have shown that the addition of either freeze-shocked starter cultures (Bartels *et al*., 1987, El-Neshawy and Groiave, 1988 and Aly, 1996) or barley extract (Zaki and Salem, 1992) to cheese milk slightly increased the levels of total volatile fatty acids in the resultant cheeses. This phenomenon may be attributed to formation of basic compounds from proteolysis, as reported previously (Novella- Rodriguez, *et al.*, 2000).

Microbial content of cheese

Table (3) shows that the additions of either freeze-shocked *L. delbrueckii subsp. helveticus* or barley extract at different levels had some stimulating effect on bacterial growth. This was more marked with increasing the level of barley extract or freeze-shocked starter culture added to the cheese. This could be attributed to the increased level of simple nitrogenous compounds in cheeses (Table 2) which simulated the growth and activity of the micro flora of the cheeses. During the storage period, the Coliform bacteria and moulds and yeasts counts of UF Feta-cheese from different treatments were absent and such results reflect the hygienic standards and sanitary condition during production. Total bacterial counts were quantitatively the dominant groups, and change of their viable numbers was significant (P≤0:05) throughout the ripening period (Manolopoulou, *et al*., 2003).

Organoleptic properties:

The results in Table (4) shows that the flavour intensity of the cheeses treated with freeze-shocked *L. delbrueckii subsp. helveticus* or barley extract were higher than in control cheese at each period of ripening. It could be also observed that the flavour of treated cheeses developed earlier than the control. This was associated with a great improvement in the body characteristics of the treated cheeses. The effect of freeze-shocked culture or barley extract was proportional to the level of added culture of barley extract. Neither flavour detects nor bitterness were detected in all treated cheeses. Statistical analysis showed that differences in total scores of Feta- cheese as affected by addition of freeze-shocked culture of *L. delbrueckii subsp. helveticus* or barley extract were acids

Table 3. Microbial analysis of UF Feta- cheese as affected by various levels of and freeze-shocked culture of *L. helveticus* and barley extract

Microbial properties (cfu /g)	Ripening period (weeks)	Feta cheese containing				
		Control	Barley Extract		Freeze-shocked culture *L. helveticus*	
			0.05%	0.10%	1%	2%
Total Bacterial Counts	Fresh	43×10^6	57×10^6	62×10^6	59×10^6	68×10^6
	2	10.6×10^6	14.2×10^6	15.4×10^6	13×10^6	16.1×10^6
	4	4.3×10^6	5.7×10^6	6.8×10^6	7.2×10^6	10.2×10^6
	8	1.9×10^6	4.4×10^6	5.5×10^6	5.1×10^6	6.3×10^6
Moulds & yeasts	Fresh	Nil	Nil	Nil	Nil	Nil
	2	Nil		Nil	Nil	Nil
	4	Nil	Nil	Nil	Nil	Nil
	8	Nil	Nil	1×10^3	Nil	Nil
Coliform-bacteria	Fresh	Nil	Nil	Nil	Nil	Nil
	2	Nil	Nil	Nil	Nil	Nil
	4	Nil	Nil	Nil	Nil	Nil
	8	Nil	Nil	1×10^3	1×10^3	Nil

Table 4. Organoleptic properties of UF Feta- cheese as affected by various levels of freeze-shocked culture of *L. helveticus* and barley extract

Ripening period (weeks)	Cheese properties	Feta-cheese containing					LSD
		Control	Barley Extract		Freeze-shocked culture *L. helveticus*		
			0.05 %	0.1 %	1 %	2.%	
Fresh	Flavour (40)	30.6	32.2	32.8	32.3	32.8	4.42
	Body & Texture (20)	15.0	15.8	15.9	15.6	15.8	2.8
	Total (100)	45.6^a	48.0^a	48.7^a	47.9^a	48.6^a	5.6
2	Flavour (40)	31.7	34.2	34.9	36.5	37.0	6.4
	Body & Texture (20)	15.9	16.5a	16.9^a	16.7^a	16.8^a	2.1
	Total (100)	47.6^b	50.7^{ab}	51.8^{ab}	53.2^a	53.8^a	4.6
4	Flavour (40)	33.0	36.9	37.0	36.5	37.0	2.8
	Body & Texture (20)	16.3	17.6	17.8	17.5	17.6	1.9
	Total (100)	49.3^b	54.5^a	54.8^a	54.0^a	54.6^a	4.5
8	Flavour (40)	34.1	38.0	38.9	38.3	36.7	2.93
	Body & Texture (20)	17.0	18.1	18.2	18.0	18.3	1.36
	Total (100)	51.1^b	56.1^a	57.1^a	56.3^a	56.0^a	4.14

Means with the same letter in the same row are not significantly different.
Significant at 0.05 level ($p\leq 0.05$).

significant ($P\leq0.05$). This could be attributed to the higher levels of soluble nitrogen, and volatile fatty which are considered to be essential contributor for flavour development. The results of present study are in agreement with those of Aly (1996) for Domiati cheese , Kebary (1996) for Ras cheese and El-Neshawy & Groiave (1988) for Bulgarian white brined cheese who reported increased flavour intensity in the previous cheeses supplemented with freeze-treated *lactobacilli*. Also, some workers have shown that incorporation of barley extract into cheese enhanced the flavour development and improved the body and texture of cheeses without causing flavour defect (Zaki and Salem 1992).

CONCLUSION

The present study demonstrated that the addition of a freeze-shocked *L. delbrueckii subsp. helveticus* or barley extract preparation to enhance cheese ripening,

increase the number of lactic acid bacteria and to improve the sensory properties of UF Feta-cheese.

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الملخص العربي

إسراع تسوية الجبن الفيتا المصنعة بطريقة الترشيح الفائق بإستخدام بادئ من *Lactobacillus delhrueckii subsp helveticus* أو بإستخدام مستخلص الشعير

خالد مغاورى الزهار

Lactobacillus delhrueckii subsp helveticus فى الصناعة. الجبن الناتج سويت لمدة شهرين على درجة حرارة ٤°م وخلال تلك الفترة درست التأثيرات الناتجة عن اختلاف البادىء المستخدم وما يتبعه من تغيرات خلال فترة التسوية ،حيث درست التغيرات فى التركيب الكيماوى والتغيرات الميكروبيولوجية ومدى قابلية المستهلك للمنتج من خلال اجراء التحكيم الحسى له. وذلك من خلال تقدير كل من الجوامد الكلية، الدهن، الحموضة الكلية، الملح، النيتروجين الكلى،النيتروجين الذائب فى الماء، النيتروجين غير البروتينى، والاحماض الدهنية الطيارة الكلية فى عينات الجبن الناتج خلال فترة التسوية. حيث صنعت الجبن من مركز اللبن (retentate) الناتج عن الترشيح الفائق والذى أدرج مع مستويات مختلفة من مستخلص الشعير وذلك إما بنسبة ٠,٠٥٪ أو ٠,١ ٪ كما صنعت باضافة سلالة من ميكروب *L. helveticus* المجمدة بالصدمة الحرارية والتى تضاف الى الـ retentate بنسبة ١٪ أو ٢٪. ولقد وجد ان إضافة مستخلص الشعير الى الـ retentate ليس له تأثير معنوى على محتوى الجبن الناتج من الدهن والرطوبة والملح والنيتروجين الكلى، ولكن وجدت زيادة معنوية فى محتوى الجبن الناتج من الحموضة الكلية. كما وجد أن أضافة سلالة *L. helveticus* إلى الراشح الناتج من الترشيح الفائق للبن والمعد لصناعة الجبن لم يؤد إلى أى تاثير معنوى فى التركيب الكيميائى للجبن الناتج. هذه الإضافات أدت إلى زيادة معنوية في تكوين المركيات النيتروجينية الذائبة، والأحماض الدهنية الحرة الطيارة الكلية، كما أدت الى تحسين النكهة والرائحة وخصائص القوام للجبن الفيتا الناتجة. وكان هذا ملحوظا بشكل أكبر في الجبن المصنع باضافة النسبة الأعلى من مستخلص الشعير عن الجبن المصنع من بادئ *L. helveticus* . الجبن المصنع من اضافة مستخلص الشعير الى الـ retentate بنسبه ١% أو باضافة ٢% من البادئ المجمد لسلالة *L. helveticus* كان أعلى فى جودته بعد أسبوعين وبعد شهر من التسوية مقارنة ببقية المعاملات الأخرى وجد أن العدد الميكروبي خلال فترة الحفظ لهذين النوعين من الجبن يزداد باضطراد ووصلت الى مستويات متماثلة باستثناء بكتيريا القولون. جميع أنواع الجبن الناتجة باستخدام البادىء المجمد أو مستخلص الشعير أظهرت نتائج مقبولة خلال التحكيم الحسى مقارنة بالجبن الفيتا التجارية.

بذلت محاولات لتحسين نوعية الجبن الفيتا المصنعة بطريقة الترشيح الفائق بأستخدام مستخلص الشعير او بادىء مجمد من