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The Optimum Conditions for Producing Fermented Milk Used in the Manufacture of Fayesh Bread

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FAYESH is one of the most popular types of bread in Egyptian kitchen, which depends on the fermentation of boiled milk using cereals and legumes. In this study, the optimum conditions for fermented milk used in the manufacture of Fayesh were evaluated. The bacteria responsible for the milk fermentation were also isolated and identified. Ten grams of whole or crushed some cereals and legumes were added separately or incombination to 150 mL beilod milk and incubated at room temperature (traditional method) or at 30, 40 and 50 ºC. The results illustrated that milk fermentation needs more time at room temperature than fermentation at 40 ºC. At 40ºC, the time of milk fermentation was the lowest compared to that at 30 and 50 ºC. Regarding the type of grains, the fermentation time was the lowest using both whole lentil (7.5 hr) and crushed wheat green (7.5 hr),followed by whole faba bean (8.0 hr).When mixing the grains, the least time for fermentation was 6.5 hr, using lentil/wheat grain mixture, followed by fababean/green wheat (7.0 hr), fababean/wheat grain (7.0 hr), green wheat/wheat grain (7.0 h), and lentil/green wheat (7.25 hr).*Staphylococcus vitulinus* was isolated from faba beans, green wheat,and wheat grain. *Staphylococcus lentus* was isolated from chickpea and sesame, while *Enterococcus faecium* was also isolated from lentil.

Keywords: Fermented foods, Milk fermentation,Fayeshbread, Cereals, Legumes.

Introduction

Indigenous fermented food such as bread, cheese, and wine, have been prepared and consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities (Madsen et al., 2001). Fermentation is cheap and the most economical technique of food production and preservation. Many types of indigenous fermented foods are made based on milk, raw products, fruits, cereals, grains, and vegetables using locally available raw materials and local knowledge (Joshi et al., 2012). The fermented foods are derived from substrates like roots, legumes, cereals, oilseeds, nuts, meat, fish, milk, palm tree, sap, etc (Uzogara et al., 1990).

Fermented foods in the Arab countries and many others in the Middle East are very typical of those found in Egypt. In Arabic and other countries, some of them have different national names in this region. One or more of these foods participate and thus provide an essential source of digestible protein in daily meals. The fermented foods are analyzed concerning their traditional manufacturing processes. It includes sour milk, Carish, Mish, Laban Zeer, Kishk, Zabady, Shamsian bread, and certain Sudanese foods that have been fermented (El-Gendy, 1983).

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The preparation of many indigenous or traditional fermented foods and beverages remains today as a house art. They are produced in homes, villages, and small-scale industries (Bol and DeVos, 1997). Traditional fermentation processes are typically uncontrolled and dependent on microbes from the environment, fermentation substrate, region geography, topography, agricultural factors, and climate to initiate it, and as such, can result in products of low yield and variable quality (Holzapfel, 2002; Ross et al., 2002 and Methaet al., 2012). Fermentation in food processing is essentially the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof under anaerobic conditions. Many societies have considered traditional knowledge and skills, which is a critical component for successful fermentation activities. Additional overarching qualities deemed useful. The level of fermentation sophistication is highly related to the level of technological development and existing institutional support systems within a region or country (Rolle & Satin, 2002). Fermentation is spontaneous as a result of competitive microbial activities. Hence some strains are best adapted with rapid growth, thereby dominating others at stages of fermentation. This microbial accounts for the succession process, as reported by many workers (Mbata et al., 2009).

Most of the traditional fermented foods are solid substrate-based, where the substrates undergo the process of natural fermentation either naturally or by adding starter cultures. Fermented foods can be described as palatable and whole foods prepared from raw or heated raw materials. They are generally appreciated for attributes such as pleasant flavor, aroma, texture, and improved cooking and processing properties. Microorganisms, by their metabolic activities, contribute to the development of characteristic features such as taste, aroma, visual appearance, texture, shelf life, and safety. Enzymes indigenous to the raw materials may play a role in enhancing these characteristics (Hammes, 1990). Traditional foods are those produced by ethnic people using locally available raw materials to make edible products that are culturally and socially acceptable to the consumer (Tamang, 2010).The traditional art of fermentation practiced by the common man continues despite the scientific and technological revolution, though remains confined mainly to rural and tribal areas, due to the inaccessibility of commercially made products in the remote regions and their cost, and the sociocultural linkages of indigenous fermented products (Thakur et al., 2004). Fermentation food can take place if there is a suitable substrate, appropriate microorganism(s) either from nature or by inoculation of specific microorganisms, and the necessary environmental conditions for the fermentation to take place (Caplice and Fitzerald, 1999).

Sour dough starter is likely the oldest, being reliant on organisms present in the grain and local environment. These starters have fairly complex microbiological makeup, and the most notable including wild yeasts, lactobacillus, and Acetobacteria in symbiotic relationship referred to as a SCOBY (Scheirlinck et al., 2008) and (Arendt et al., 2007). They are usually maintained over long periods. For example, the Boudin Bakery in San Francisco has used the same starter dough for over 150 years. Mother dough often refers to a sourdough, and in this context, the term starter often refers to all or a piece of mother dough (Renneberg and Demain, 2007). However, mother dough may also apply to a first-generation yeast sponge (Esposito, 2003) so the process (Hui et al., 2006) used concerning the ingredients and fermentation times is essential to understanding yeast versus sourdough methods. A roughly synonymous term used in French baking is Chef (Calvel, 2001).Fayish bread is a traditional fermented food in Upper Egypt, which is produced at the household level and prepared during marriage ceremonies, religious and served with tea. Fermenting starter of Fayesh bread prepared from mixed cereals and legumes with milk. Relatively little information is available on the starter culture of Fayesh. In Upper Egypt, Fayesh bread manufactured using fermented milk by adding a cup of boiling milk to 2 table spoons of faba bean, lentil, greenwheat "Freekeh", chickpea, sesame and wheat placed in a jar, sleeve, wrapped in towel or cloth and leave in a warm dark place for two days or less. Therefore, this work aims to evaluate the optimal conditions for producing fermented milk using some cereals or legumes as a starter used in the manufacture of Fayesh bread, as well as isolating and identifying the microbial strains responsible for this fermentation.

Materials and Methods

Materials

- Cereals and legumes such as fababean (*Vicia faba*), lentil (*Lens culinaris*), green wheat "Freekeh" (*Triticum aestivum*),chickpea (*Cicer arietinum*), sesame (*Sesamum indicum*) and wheat

(*Triticum aestivum*) were obtained from local markets, Sohag, Egypt. Milk was obtained from animal production Farm, Faculty of Agriculture, Sohag University Sohag, Egypt. The average composition was 87.20, 3.55, 3.75. 4.75 and 0.75% for moisture, proteins, fat, carbohydrates and ash, respectively.

Methods

Fermentation process

The fermentation process was carried out by adding 10 g of faba bean, lentil, green wheat (Freekeh), chickpea, sesame, and wheat grains separately or in combination, as whole grains or crushed grains, to 150 mL of boiled milk after cooling in an airtight container. The containers were left to ferment by its microflora at room temperature (~ 28 °C, traditional method) or a 30, 40 and 50°C until the fermentation process was completed (milk separated into two layers;serum layer, and foamed fermented layer). The time of fermentation was measured and expressed as a hour.

Chemical composition of raw materials

Moisture, protein, fatand ash contents of cereals, legumes and milk were determined according to the methods of AOAC (2007). The total carbohydrate content was calculated by difference.

Microbiological analysis

The total bacterial counts were determined using the plate counts technique on a nutrient agar medium to enumerate different viable microorganism groups according to procedures by APHA (1976) and Difco (1984). The plates were incubated at 37 °C for 48 hr. All tests were performed in duplicate, expressing the results as log colony-forming units (CFU)/g of the product.

Microbial isolation and enumeration

Isolation and cultivation of bacteria

One gram was weighted from each fermented sample, added to 100 mL sterilized distilled water in 150 mL conical flask, and then stirred (bioMérieux Inc., Hazelwood, MO,USA) for 15 min at a speed of 200 rpm/min (Janssen et al., 2002). The stirred sample was also serially diluted to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}, and 200 µL of each last three dilutions was used to inoculate each of triplicate plates at each dilution level to constitute a counting set of 9 plates. Inoculate were spread over the surface of the Nutrient-agar medium using sterile glass spreading rods (Davis et al., 2005). All plates were incubated at 30°C for 2 days in sealed polyethylene bags (40-µm film thickness). Morphologically distinct colonies were purified and maintained on nutrient agarslant at 4 °C.

Screening and characterization of strain identification

Morphological and physiological test

The ability of the bacterial isolates from faba bean, lentil, green wheat (Freekeh), chickpea, sesame, and wheat to hydrolyze urea, arginine, and Polymyxin B resistance were examined. The morphological and physiological characteristics of the bacterial isolates were compared with the data from Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

Metabolic profiling

The Vitek 2 compact Automated Expert System (AES) (bioMérieux Inc., Hazelwood, MO,USA) was used for metabolic profiling. Using the Vitek 2 ID-GP card (bioMérieux), identification of Gram-positive cocci occurs through testing the organism's metabolic activity in 43 biochemical tests designed to measure carbon source utilization and enzymatic activity, the reference identification was determined with API Staph and API 20 Strep identification kits. Overall, the VITEK 2 GP correctly identified 96.5% of the isolates, including 2.2% low discrimination with the correct species listed. Misidentifications occurred at 3.3%, and no identifications occurred at 0.2%.The GP identification card is based on established biochemical methods and newly developed substrates (Atlas, 1993; Barros et al., 2001; Billeet al., 1992; Collins &Lawson, 2000 and Holt et al., 1994).

Database development

The databases of the VITEK 2 identification products are constructed with large strain sets of well-characterized microorganisms tested under various culture conditions. These strains are derived from a variety of clinical and industrial sources as well as from the public (*e.g.,* ATCC) and university culture collections.

Analytical techniques

Test data from an unknown organism are compared to the respective database to determine a quantitative value for proximity to each of the database taxa. Each of the composite values is compared to the others to determine whether the data are sufficiently unique or close to one and more of the other database taxa. If a unique identification pattern is not recognized, a list of possible organisms is given, or the strain is determined to be outside the scope of the database.

Statistical analysis

Data was analyzed by analysis of variance (MANOVA) using the GLM procedure with SAS software (2008). Significant differences were determined as P ≤ 0.05 using Duncan's multiple range tests.

Results and Discussion

Composition of cereals and legumes

The type of microflora in all food fermentations depend on the pH, water activity, temperature, salt concentration, and chemical composition of the food matrix (Blandino et al., 2003). Thus, the composition of cereals and legumes (faba bean, lentil, green wheat "Freekeh," chickpea, sesame, and wheat), used in the making of Egyptian Fayesh bread were determined (Table 1). Faba bean had the highest protein content (31.33%, $P < 0.05$), followed by lentils (26.44%, $P < 0.05$), chickpea (20.58%), and Sesame (18.58%), but wheat and green wheat had the lowest protein content (12.86 and 12.40%, $P < 0.05$), respectively. Carbohydrates content was higher in both green wheat and wheat (73.48 and 72.44 %) followed by chickpea, lentil, and faba bean, while was lower in sesame (22.92%). However, Sesame had the highest fat (46.83%) and ash (5.57%) content ($P < 0.05$)than other cereals and legumes. These values were close to those mentioned by Carsanba et al. (2017) in green wheat, Nzikou et al. (2009) in sesame seeds, and Fišteš et al. (2014) in Chickpea. Additionally, the protein and carbohydrates content of lentil was in the normal range (26.6-30.4% and 48.3-61.0%, respectively), reported by Hefnawy (2011).

Raw materials and fermentation process

Initial experiments indicate that the use of sterilized cereals or legumes, using autoclave at 121°C for15 min, with boiled milk after cooling, had no positive effect on the fermentation process compared to the non-sterilized cereals or legumes. These results explained that the fermentation process was done by endoenzymes or the original microflora on the surface of cereals or legumes. These results in the same line with (Harlander, 1992) who reported that microbes responsible for the fermentation might be the natural microflora indigenously present on the substrate, or they may be added as starter cultures. The addition of milk to the cereal and legumes stimulates the endoenzyme into action and growth of microbes (Achi and Ukwuru, 2015). Figure 1 illustrates the success of fermentation of boiled milk using unspecialized grains by separating the milk into two layers; serum layer and foamed fermented layer.

Fig. 1. Images of fermentd milk by using unsterilized cereals or legumes.

1- Fermented milk using crushed green wheat "Freekeh"
2- Fermented milk using whole wheat
3- Fermented milk using crushed faba bean
4- Fermented milk using whole chickpea
5- Fermented milk using whole lentils

Milk fermentation using whole cereals and legumes at different temperatures

Traditional Egyptian Fayesh bread is made by adding fermented milk using cereals or legumes at room temperature in a warm place, as a starter, to Fayesh bread dough. The effect of the incubation temperature on the time required to milk fermentation using whole cereals and legumes is presented in Table 1. In general, the period of milk fermentation at 40º C was the lowest, while fermentation at 30 ºC was the longest ($P < 0.05$).This means the incubation at 40º C was more suitable for milk fermentation with whole cereals and legumes; except green wheat, milk fermentation was the lowest at 50º C (7.0 h).Regarding the type of grains, the fermentation period was the lowest with whole lentils and chickpea as ($P < 0.05$) followed by faba bean at 40 and 50º C, while the lowest was with lentils and chickpea followed by faba bean at 30ºC.($P < 0.05$).

Also, the incubation of green wheat "Freekeh" with chilled boiled milk at 30, 40 and 50º C, decrease the fermentation time to 9, 8 and 7 hr ($P < 0.05$), respectively. There is a difference between the samples incubated on high-temperature degrees and those equipped with the normal room temperature. Using high temperature leads to accelerating the starter production compared with room temperature. On the contrary, using sesame and wheat grains in milk fermentation exhibited the longest fermentation period compared to other cereals and legumes at different temperatures ($P < 0.05$). The increased fermentation period with sesame may be due to the high fat content (Table 1).These results are agreement with those reported by Caplice and Fitzerald (1999). Environmental conditions, like temperature and moisture, need to be optimum for a specific fermentation, as well as the intrinsic factors of fermentation, including the pH, type of sugar, nutrients, availability or otherwise of oxygen, etc. The composition and quality of the raw materials, the microflora involved, the amount of water, type of raw material, and time also influence the fermentation. Also, the type of organisms that are found in association with each type of fermentation depends on ecological parameters like temperature, pH, inoculums, size, and nature of cereal and propagation time. Hence, fermenting organisms are not readily substrate-specific but are conditioned by the factors mentioned (Hammes et al., 2005).

Milk fermentation using crushed cereals and legumes at 40 ºC and room temperatures

Based on the above results, 40ºC was the best incubation temperature for milk fermentation using whole cereals and legumes, the use of crushed cereals and legumes in the fermentation of milk was compared between 40 ºC and room temperature (`25-30ºC). The time recorded for complete milk fermentation using crushed cereals and legumes was shown in Table 3. At 40ºC, using whole faba beans and lentils in milk fermentation was better than crushed grains, while crushed wheat grains, sesame, chickpea, and wheat were the best ($P < 0.05$). Compared to room temperature, milk fermentation using crushed cereals and legumes was better than that at 40 ºC. However, using crushed green wheat "Freekeh" was the best (7.5 hr) at 40 ºC. The time of milk fermentation were 8.5, 8.5, 7.5, 8.5, 9.0 and 8.5 hr using faba beans, lentils, green wheat, lentils, sesame and wheat, respectively. At room temperature, the time for fermentation with crushed Faba beans and green wheat was the lowest (9.5 hr), while the fermentation period was the longest ($P < 0.05$) when using crushed lentils and sesame seeds (13-14 hr). These results agree with Achi and Ukwuru (2015) who reported that reduction of size through milling and sometimes the use of specific microorganisms and enzyme actions enable fermentation to start (Caplice and Fitzerald, 1999). The microflora which may be indigenously present on the substrate, or added as a starter culture (Blandino et al., 2003), or maybe present in or on the ingredients and utensils, or in the environment are selected through adaptation to the substrate and by adjusting the fermentation conditions (Soni and Sandhu, 1990).

Milk fermentation using the cereals and legumes alone or incombination at 40 ºC and room temperature

Some peoples sometimes prepare the fermented milk for making Egyptian Fayesh bread using mixtures of two types of cereals or legumes to improve the fermentation process and decrease the fermentation period. Regarding the added quantity, the time of milk fermentation decreased on the addition of faba bean, lentil, chickpea, or wheat ($P <0.05$), up to 20 g both alone; the decreasing was more pronounced with lentil, from 8.0 to 7.5 hr (Table 3). On the contrary, the time of fermentation was not affected by the increase in the amount of sesame, while it increased with the increase of green wheat from 7.5 to 8.5 hr ($P <0.05$).

TABLE 1. Chemical composition of cereals and legumes used in milk fermentation.

Raw materials	Moisture (%)	Protein (%)	Fat (%)	Total Carbohydrates (%)	Ash (%)
Faba bean	7.44 ^{d}	31.33 ^{a}	1.45 ^{d}	56.70^{d}	3.08 ^{c}
Lentils	8.22 ^{c}	26.44^{b}	1.30 ^{d}	60.69^{c}	3.35 ^{b}
"Greenwheat "Freekeh	10.20 ^{b}	12.40^{e}	2.09 ^{c}	73.48^{a}	1.83 ^{d}
Chick pea	6.68 ^{e}	20.58 ^{c}	5.80^{b}	63.67^{b}	3.57 ^{b}
Sesame	6.10 ^{e}	18.58^{d}	46.83 ^{a}	22.92 ^{f}	5.57 ^{a}
Wheat grains	11.20 ^{a}	12.86 ^{e}	1.90^{d}	72.44^{a}	1.60^{f}

Means (n=3) with the same small letters in the same column are not significantly different at P <0.05.

TABLE 2. Effect of whole cereals and legumes on the period of milk fermentation at different temperature.

Cerealsand legumes	Fermentation period (h) at different temperature		
	30ºC	40 ºC	50 ºC
Whole faba beans	10.0 ^{Ad}	8.0^{Ce}	9.5^{Bb}
Whole lentils	9.0^{Ae}	7.5 ^{Cd}	8.0^{By}
Whole green wheat "Freekeh"	9.0^{Ae}	8.0^{Bc}	7.0^{Cd}
Whole chickpea	11.5^{Ab}	9.0^{Cb}	10.0^{Ba}
Whole sesame	11.0 ^{Ac}	10.0 ^{Ba}	10.0 ^{Ba}
Whole wheat grains	12.0^{Aa}	10.0 ^{Ba}	10.0 ^{Ba}

Means (n=3) with the same capital letters in the same rowor the same small letters in the same column are not significantly different at P <0.05.

TABLE 3. Effect of crushed cereals and legumes on the period of milk fermentation at 40 ºC and room temperature (25-30ºC).

Cereals and legumes	Fermentation time (h)	
	Room temperature	40 ºC
Crushed faba beans	9.5 ^{Ad}	8.5 ^{Bf}
Crushed lentils	14.0^{Aa}	8.5 ^{Bf}
Crushed green wheat "Freekeh"	9.5 ^{A}	7.5^{Bg}
Crushed chickpea	10.0 ^{A}	8.5 ^{Bf}
Crushed sesame	13.0 ^{A}	9.0 ^{Be}
Crushed wheat grains	10.0 ^{A}	8.5 ^{Bf}

Means (n=3) with the same capital letters in the same rowor the same small letters in the same column are not significantly different at P <0.05.

When mixing seeds, there are significant differences (P > 0.05) in the time of fermentation, depending on the type of mixture and temperature of incubation. Using lentil/wheat lowers the time of milk fermentation (6.5 hr), followed by faba bean/green wheat, faba bean/wheat or green wheat/wheat grains, lentil/sesame, chickpea/wheat, crushed faba bean/sesame or sesame/wheat grains (7.0, 7.0, 7.5, 7.5, 8.0 or 8.0 hr, respectively). The use of sesame alone or with wheat grains led to delaying the formation of the fermenting starter (P <0.05). This effect may be since sesame is rich in oil that may hinder the growth of microbes on the sesame surface responsible for fermenting the starter. On the other hand,the fermentation process was better at 40 ºC than at room temperature (P < 0.05). The fermentation period ranged from 9.5 to 17 hr at room temperature, while it ranged from 6.5 to 9 hr at 40 °C. However, using 20 g of faba bean or green wheat showed the lowest time of fermentation at room temperature (9.5 hr) compared to other grains or their mixtures. This means that increasing the weight of cereals or legumes alone or in combination, > 10 g, does not reduce the time of fermentation at room temperature.

TABLE 4. The effect of the cereals and legumes alone (20 g) or incombinatios on the period of milk fermentation at 40 ºC and room temperature (25-30ºC.

Samples	Treatments	Fermentation time (h) at 40 ºC	Fermentation time (h) at room temperature
Faba bean	20 g faba bean	8.0^{Bb}	9.5^{h}
	10 g faba bean + 10 g green wheat “Freekeh”	7.0^{Bg}	12.0^{f}
	10 g faba bean + 10 g lentil	7.5^{Bd}	12.0^{Af}
	10 g faba bean + 10 g chickpea	7.45^{Be}	13.0^{Ae}
	10 g faba bean + 10 g sesame	8.0^{Bb}	12.0^{Af}
	10 g faba bean+ 10 g wheat	7.0^{Bg}	13.0^{Ae}
Lentil	20 g lentil	7.5^{Bd}	14.0^{Ad}
	10 g lentil + 10 g green wheat “Freekeh”	7.25^{Bf}	15.0^{Ac}
	10 g lentil + 10 g chick pea	8.0^{Bi}	17.0^{Aa}
	10 g lentil + 10 g sesame	7.5^{Bd}	16.5^{Ab}
	10 g lentil + 10 g wheat	6.5^{Bh}	14.0^{Ad}
Green wheat “Freekeh”	20 g green wheat “Freekeh”	8.0^{Bc}	9.5^{Ah}
	10 g green wheat “Freekeh” +10 g chickpea	8.5^{Bb}	10.0^{Ag}
	10 g green wheat “Freekeh” +10 g sesame	8.5^{Bb}	14.0^{Ad}
	10 g green wheat “Freekeh” + 10 g wheat	7.0^{Bg}	12.0^{Af}
Chickpea	20 g chickpea	9.0^{Ba}	10.0^{Ag}
	10 g chickpea + 10 g sesame	8.5^{Bb}	13.0^{Ae}
	10 g chickpea + 10 g wheat	7.5^{Bd}	14.0^{Ad}
Sesame	20 g sesame	9.0^{Ba}	13.0^{Ae}
	10g sesame + 10 g wheat	8.0^{Bc}	15.0^{Ac}
Wheat	20 g wheat	9.0^{Ba}	10.0^{Ag}

Means (n=3) with the same capital letters in the same rowor the same small letters in the same column are not significantly different at $P < 0.05$.

Total bacterial countof fermented milk

Table 5 shows the total bacterial count of fermented milk using cereals and legumes. The total bacterial count was the highest in fermented milk with whole wheat grains (5.70 $\log_{10}$ CFU/g) but was the lowest in fermented with sesame (4.07 $\log_{10}$ CFU/g). The remaining treatments had mediumtotal bacterial count, which ranged between 4.23 to 4.84 $\log_{10}$ CFU/g.These results are in the same line with Harlander (1992) who reported that microbes responsible for the fermentation might be the natural microflora indigenously present on the substrate, or they may be added as starter cultures.

TABLE 5. Total bacterial count of milk fermented using cereals and legumes at 40 ºC.

Cereals and legumes	Total bacterial count ($\log_{10}$cfu/g)
Fababeans	4.23^{c}
Lentils	4.69^{b}
Green wheat “Freekeh”	4.65^{b}
Chickpea	4.84^{b}
Sesame	4.07^{c}
Wheat grains	5.70^{a}

Means (n=3) with the same small letters in the same column are not significantly different at $P < 0.05$.

TABLE 6. Biochemical characterization of bacteria isolated from fermented milk using cereals and legumes.

No.	Test	Samples	Faba bean	Lentils	Green-wheat "Freekeh"	Chick-pea	Sesame	Wheat grains
		Isolates	*Staph. vitulinus*	*Enterococcus faecium*	*Staph. vitulinus*	*Staph. lentus*	*Staph. lentus*	*Staph. vitulinus*
	D-Amygdalin		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	phosphatidylinositol phospholipase c		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	D-Xylose		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
	Arginine dihydrolase		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	Beta galactosidase		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	Alpha glucosidase		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Ala-Phe-pro-arylamidase		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Cyclodextrin		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	L-aspartic acid arylamidase		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	beta glucopyranosidase		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Alpha-mannosidase		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	phosphatase		+Ve	-Ve	+Ve	+Ve	+Ve	+Ve
	leucinearylamidase		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	l-prolinearylamidase		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Beta-Glucorinidase (0.018mg)		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Alpha-Galactosidase		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	l-pyrrolidonyl-arylamidase		-Ve	+Ve	-Ve	-Ve	-Ve	-Ve
	Beta-Glucorinidase (0.0378 mg)		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Alanine arylamidase		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	Tyrosine arylamidase		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	D-Sorbitol		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	Urease		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	Polymyxin B resistance		-Ve	+Ve	-Ve	-Ve	-Ve	-Ve
	D-galactose		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	D-ribose		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
	l-lactate alkalization		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	Lactose		-Ve	+Ve	-Ve	-Ve	-Ve	-Ve
	N-acetyl-glucosamine		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	D-Maltose		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	Bacitracin resistance		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
	Novobiocin resistance		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	Growth in 6.5 % NaCl		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
	D-Mannitol		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
	D-Mannose		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
	Methyl B-D-glucopyranoside		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
	pullulan		-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
	D- Raffinose		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	O 129 resistance (comp. vibrio.)		-Ve	+Ve	-Ve	+Ve	+Ve	-Ve
	Salicin		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
	Sucrose		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
	D- Trehalose		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
	Arginine dihydrolase		-Ve	+Ve	-Ve	-Ve	-Ve	-Ve
	optochin test resistant		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve

Isolation and characterization of bacteria isolated from fermented milk

From each fermented sample, one bacterial strain, which is the cause of success fermentation, was isolated and identified by Vitek 2 compact Automated Expert System (AES) was used for metabolic profiling. Using the Vitek 2 ID-GP card The morphological and physiological characteristics of the bacterial isolates were compared with the data from Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). The results exhibit that *Staphylococcus vitulinus* isolated from faba bean, green wheat "Freekeh and wheat, *Enterococcus faecium* isolated from lentils, and *Staphylococcus lentus* isolated from chickpea and sesame. *Staphylococcus vitulinus* and *S. pulvereri* were first described as novel *Staphylococcus* spp. in 1994 (Webster et al., 1994) cited in Nam et al. (2012). *Staphylococcus lentus* is a member of the *Staphylococcus sciuri* group, which are Gram-positive, oxidase-positive, and coagulase-negative cocci, *Staphylococcus lentus* F1142, was isolated from doenjang, which is a Korean soybean paste (Nam et al., 2012). *Enterococci*, especially *Enterococcus faecalis* and *Enterococcus faecium*, have been studied in Gaborone, Botswana for the occurrence, and antibiotic resistance of milk, beef, and chicken. *Enterococci* were isolated from these sources using API 20 Strep kits with bile esculin agar (Chingwaru et al., 2003).

Conclusion

Fayesh is one of the most famous Egyptian breads which have extended to all Arab kitchens. It mainly depends on using fermented milk using cereals and legumes at room temperature (~28 ºC). we can conclude that 40 ºC was the optimum temperature for milk fermentation using whole or crushed cereals or legumes. The best cereals and legumes (whole or crushed) for milk fermentation are faba bean, lentil, green wheat, and chickpea. Increasing the added amount of cereals or legumes, > to 10 g, or mixing them does not significantly improve the time of milk fermentation ($P < 0.05$). *Staphylococcus vitulinus*, *Staphylococcus lentus*or *Enterococcus faecium*,which have been isolated from fermented milk, may have been responsible for the fermentation process. In the future, we need other studies to activate these strains and adding them as starter cultures, as well as to study the optimal conditions for these cultures and the properties of the resultant product.

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