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PHYSICOCHEMICAL ANALYSIS AND MICROBIAL EVALUATION OF BUTTER SOLD IN ASSIUT CITY, EGYPT

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ABSTRACT

In this aspect, this study aimed to evaluate the quality of pasteurized and cooking (Falahy) butter in Assiut City, Egypt. A total of random 160 pasteurized and Falahy butter samples (80 samples each) were bought from different localities in Assiut city. The sensory assessment for pasteurized and Falahy butter showed that 78.75, 62.5% of samples exceeded a score of 90 (excellent) and 21.25, 37.5% had a score (80-89). The mean values of peroxide index, TBRA reactive substances and acid index were (0.39±0.02, 0.44±0.03 meq/kg), (0.46±0.03, 0.51±0.02 mg MDA/Kg) and (0.67±0.01, 0.82±0.02 mg KOH/g) for pasteurized and Falahy butter samples, respectively which revealed that all samples were acceptable with the absence of both hydrolytic and oxidative types of rancidity. The mean values of the microbiological evaluation were ($1.3\times10^5 \pm 1.6\times10^4$, $8.8x10^6 \pm 1.3\times10^4$), ($1\times10^5 \pm 2.3\times10^4$, $3.2\times10^5 \pm 3.3\times10^4$), ($5.7\times10^4 \pm 1.3\times10^4$, $9.4\times10^4 \pm 2\times10^4$), ($1.5\times10^4 \pm 3\times10^3$, $2.8\times10^4 \pm 4.5\times10^3$) and ($1.3\times10^3 \pm 3.3\times10^2$, $1.6\times10^4 \pm 4.2\times10^3$) CFU/g for the total colony, psychrotrophic, lipolytic, yeast and mold counts in pasteurized and Falahy butter samples, respectively. The results revealed the substandard production and storage conditions which call for improvement of butter production modern technologies and awareness creation about the hygienic production, processing and handling of butter.

Keywords: Pasteurized butter; Falahy butter; Peroxide value; TBRA; Acid value; Microbiological assessment.

INTRODUCTION

In the sphere of food production, consumer demands have shifted dramatically. Consumers are becoming that food contributes directly to their health (Mollet and Rowland, 2002). Food is now designed to not only satisfy hunger and provide essential nutrients for humans, but also to prevent nutrition-related diseases and improve consumers' physical and emotional well-being (Menrad, 2003). Carbohydrates, proteins, fat, and oils are the main components of food products. Nowadays plant oils greatly reduce animal fat consumption in the diet, nevertheless, the benefits of animal fats especially milk fats can't be denied. Fat is a macronutrient that humans require in relatively substantial

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amounts as a source of energy and fatty acids, a heat conserver, a component of cell walls, and a delivery vehicle for fat-soluble vitamins A, D, E, and K, which act as insulators and shock absorbers for the body.

Milk and dairy products are one of the most important groups in the daily food pyramid. Economical, commercial, quality, and safety aspects of these products are very important (Tvrzicka *et al*., 2011). The hygienic quality of milk products is necessary to provide the consumer with safe, wholesome, and high-quality products.

Butter provides a high nutritional value to customers, but it is also a product that is susceptible to various types of spoilage due to specific factors (atmospheric oxygen, high temperature, light, heavy metal ions), which alter its sensory characteristics and, ultimately, lead to pathological changes in the human body. Since butter is one of the most popular varieties of dairy products in Egypt, and of a higher nutritional value, it constitutes a public health hazard (Henin and Kaldes, 1992). Microbiological concerns such as cheesy, putrid, or fruity odours, as well as rancid flavour caused by hydrolysis, can cause the butter to spoil (Rady and Badr, 2003). Many Psychrotrophic strains of bacteria, yeasts, and molds have been implicated in spoilage and lipolysis of butter at temperatures below 5°C and some even below 0°C (Collins *et al*., 1989).

The aim of this study is to describe the sensory, microbiological, and physicochemical properties of some pasteurized and cooking (Falahy) butter sold in Assiut city.

MATERIALS AND METHODS

1. Collection of samples:

A total of 160 random samples of pasteurized butter and traditionally manufactured butter (Falahy butter). 80 samples each were collected from different localities in Assiut city, Egypt (supermarkets, dairy shops, street vendors and different farmers' houses). Samples were collected under complete aseptic conditions in a sterile plastic bag and each sample (100-150 g) was put in an ice tank with a thermometer to maintain the temperature at 4° C. Each butter sample was divided under aseptic conditions into two subsamples; the first was kept in the refrigerator at 8°C for sensory and chemical examinations, while the second part of the samples was transferred in iceboxes soon as possible to the laboratory for microbiological examination.

2. Evaluation of butter quality:

2.1. Sensory analysis according to (IS, 2003):

A group of 9 non-smoker panelists in the Animal Health Institute joined to judge samples and a description, a scale sheet was prepared for grading the examined samples. A grade point was given towards the quality of each sample with the numerical value assigned to each factor. The sum of the evaluation of all essential factors which give a theoretically perfect product together with the defects encountered is discussed under the items on the score card.

2.2. Chemical analysis of the samples took place at Central Laboratory, Faculty of Veterinary Medicine, Assiut University, Egypt using a UV-2100 spectrophotometer. Each sample was mixed thoroughly and the tested samples were softened and melted in test sample containers, by warming in a water bath maintained at 37-39°C. Samples were shaken vigorously to obtain a homogeneous fluid emulsion free from unsoftened pieces. For Falahy butter clear supernatant fat was filtered through dry filter paper in a hot funnel.

Estimation of peroxide value according to IDF (2006), Thiobarbituric acid reactive substances (TBARS) assay according to Ke and Woyewoda (1979), and Estimation of Acid value according to ISO (660:2005).

2.3 Microbiological evaluation

Samples preparation and serial dilutions were made according to ISO (6887-1:2017), and then subjected to the following examinations.

Total bacterial count according to ISO (4883-2:2013) standard plate count technique was used for counting the total bacterial content of butter samples. Appropriate dilutions of butter samples were plated in duplicate on standard plate count agar medium.

Psychrotrophic colony count was carried out using plate count agar after incubation

at 7 °C ± 1 ° C for 10 days according to ISO (17410:2019).

Enumeration of lipolytic microorganisms was carried out by surface plating technique onto LB medium with addition of egg-yolk emulsion then incubation at 37 °C for 24-48 hours (Gubash, 1991).

Total molds and yeasts count according to Bailey and Scott (1998) using Sabouraud dextrose agar then incubation at 25°C for 5 days.

2.4. Statistical analysis:

The statistical program Graph pad Prism 5 (version 5.01) was used for data analysis (Prism 5, 2007). Then described statistics of ANOVA were performed to measure the mean ± standard error (SE). Differences between concentrations were assessed by the Dunnett Multiple Comparison test ($P < 0.05$).

RESULTS

Table 1: Overall acceptability of butter samples based on their organoleptic inspection

Grade	Pasturized butter		Falahy butter	
	No. 80	%	No. 80	%
Excellent (A) score:(90 or more)	63	78.75	50	62.5
Good (B) score (80-89)	17	21.25	30	37.5

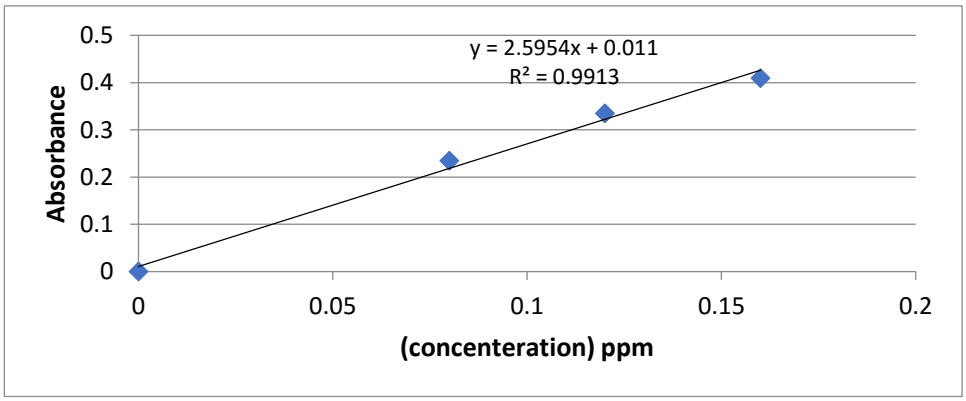

Figure 1: A linear regression curve of standard concentration of peroxide value with a correlation coefficient of 0.9913 and regression equation of $y = 2.5954x + 0.011$. Each point in the regression represents the replicate measurement ($n = 3$).

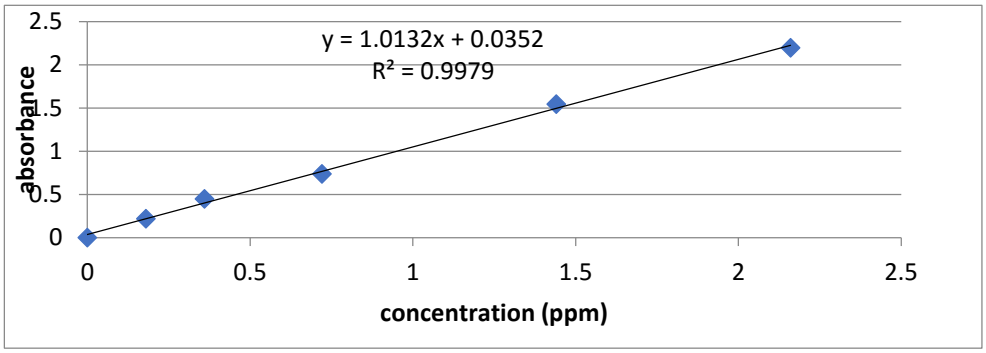

Figure 2: A linear regression curve of standard concentration of TBRA value with a correlation coefficient of 0.9979 and regression equation of $y = 1.0132x + 0.0352$. Each point in the regression represents the replicate measurement ($n = 3$).

Table 2: Mean of chemical properties of butter samples.

Analysis	Pasteurized butter (Counts (CFU/g)					Falahy butter (Counts (CFU/g)				
	Min.	Max.	Mean± SEM	Positive samples		Min.	Max.	Mean± SEM	Positive samples	
				No.80	%				No.80	%
TBC	$1x10^2$	$8x10^5$	$1.3x10^5 \pm 1.6x10^{4*}$	80	100	$1.5x10^3$	$4.8x10^6$	$8.8x10^6 \pm 1.3x10^{4*}$	80	100
Psychrotrophic count	$1x10^2$	$9x10^5$	$1x10^5 \pm 2.3x10^{4*}$	72	90	$5x10^2$	$9.5x10^5$	$3.2x10^5 \pm 3.3x10^{4*}$	76	95
Lipolytic count	$1x10^2$	$7x10^5$	$5.7x10^4 \pm 1.3x10^4$	72	90	$2x10^2$	$9x10^5$	$9.4x10^4 \pm 2x10^4$	74	92.5
Yeast count	$1x10^2$	$1.4x10^5$	$1.5x10^4 \pm 3x10^{3*}$	63	78.75	$1x10^2$	$1.9x10^5$	$2.8x10^4 \pm 4.5x10^{3*}$	66	82.5
Mold count	$1x10^2$	$3x10^4$	$1.3x10^3 \pm 3.3x10^{2*}$	28	35	$2x10^2$	$2x10^5$	$1.6x10^4 \pm 4.2x10^{3*}$	37	46.25

*Significant change ($P < 0.05$) between Pasteurized and Falahy butter samples

Table 3: Statistical analytical results of different microbial groups/g of examined butter samples.

Analysis	Pasteurized butter			Falahy butter		
	Min.	Max.	Mean± SEM	Min.	Max.	Mean± SEM
peroxide value (mM O2/kg fat)	0.1	0.9	0.39±0.02	0.1	1.3	0.44±0.03
TBRA value (mg MDA/Kg)	0.04	0.9	0.46±0.03*	0.09	0.88	0.51±0.03*
Acid Value (Mg KOH/g)	0.44	0.56	0.67±0.01*	0.56	1.2	0.82±0.03*

*Significant change ($P < 0.05$) between Pasteurized and Falahy butter samples

DISCUSSION

Butter is one of the most often consumed fats in Egyptian households. In Egypt, there are Pasteurized packaged butter and Falahy butter that are produced in villages by rural women that are usually using their traditional knowledge during manufacturing (Meshref, 2010). Although the butter is not a highly putrescible product, it does undergo spoilage by bacteria and molds. Butter is generally marketed wholesale according to its score, or ~~to~~ its grade. Color, body and texture, salting, flavor, and packaging are considered the basics of butter evaluating, scoring, or judging. Results recorded in Table 1 showed the overall acceptability of examined butter samples based on their organoleptic examination which revealed that 78.75% (63 samples) of pasteurized butter and 62.5 % (50 samples) of the examined Falahy butter samples had the final score of 90 or more with grade A (excellent), 21. 25 % (17 samples) of pasteurized butter samples and 37.25 % (30 samples) of examined Falahy butter samples had the final score of 80-89 with a Grade B (Good).

Relatively the obtained results had higher scores than El-Mossalami and Abdel-Hakem (2014) where the sensory evaluation of the 25 butter samples collected from Alexandria city, showed that 40, 36, and 24% of samples were graded as good, fair, and poor, respectively. Also, scores obtained by El-Magraby (2015) revealed that 10% were graded AA, 6.67 % as A, 26.67 % as B, and 56.66 % as C.

During the storage period of butter, rancidity had been formed due to oxygen, light, water, heat, enzymes, and microorganisms. The production of peroxide and hydroperoxides happens when the unsaturated fatty acids with double bonds react with oxygen, causing oxidation in the fats. Saturated and unsaturated aldehydes, hydrocarbons, alcohols, unsaturated ketones, and malonaldehydes are degradation products of hydroperoxides (El-Hadad and Tikhomirova, 2018).

Degradation tests, such as peroxide values (PV) and thiobarbituric acid (TBA) tests are often used to determine how long butter may be stored without losing its edible characteristics and to determine the amounts of hydrolyzation and oxidation in milk fat.

Peroxide is the primary oxidation product (Erkaya *et al*., 2015) and PV decreases with advanced oxidation. The threshold value for the sense of oxidative rancidity can be accepted as 3.00 mEq O_2/kg in butter (Altun *et al*., 2011). In Table 2 the PVs of the examined samples were between 0.1 and 0.9 mEq O_2/kg in pasteurized butter with an average of 0.39±0.02 meq O_2/ kg which was like the results obtained by Koyuncu and Tuncturk (2017) and Méndez-Cid *et al.* (2017). But higher than the results of El-Safty *et al*. (2017), Laikoja *et al*. (2017) and Silva *et al*. (2019) and lower than that recorded by Akgül *et al*. (2021) and Najgebauer-Lejko *et al*. (2009). While for Falahy butter samples, the PVs were found to be 0.1 to 1.3 mEq O2/kg in Falahy butter samples with an average of 0.44±0.03 meq O2/ kg The obtained result was like that recorded by Ramadan *et al*. (2009) and Simsek (2011) but higher than the results of Idou *et al.* (2010) and Asdagh and Pirsa (2020) and lower than the results of Abid *et al*. (2017) and Hassanzadazar *et al*. (2017).

It is worth mentioning that no significant differences were observed between peroxide values of pasteurized and Falahy butter in this study which were in good agreement with the

findings given by Veberg *et al*. (2006) and Samet bali *et al.* (2009), who indicated that fresh butter showed an only very low concentration of peroxides.

The increase in peroxide value could be attributable to fatty acid oxidation caused by a variety of variables such as high temperatures, packing in containers impermeable to light, and loose locks in addition to poor storage and poor handling (Ibrahim *et al*., 1986).

Degradation of the hydroperoxides to malonaldehydes is possible as oxidation increases due to the long storage period. Because the peroxide test cannot detect malonaldehydes, TBA analysis is recommended to determine oxidative deterioration.

The results listed in Table 2 show that the TBA of pasteurized butter samples was between 0.04 and 0.9 with an average of 0.46±0.03 mg MDA / Kg. These results were higher than that obtained by El-Safty *et al.* (2017), Koyuncu and Tuncturk (2017) and Méndez-Cid *et al*. (2017). For Falahy butter samples, the TBA was between 0.09 and 0.88 with an average of 0.51±0.02 mg MDA / Kg which is higher than that recorded by Ramadan *et al.* (2009) and Simsek (2011).

The level of fat oxidation, as measured by peroxide values and TBARS, varied the most between samples due to differences in storage time, the influence of air, light, storage temperature, the acidity of the cream at the time of churning, ferments, and enzymes, salt, metals as catalytic oxidising agents, and organic compounds as oxidation inhibitors, as well as salt, metals as catalytic oxidising agents and organic compounds as oxidation inhibitors (Zaptalov *et al*., 2015). Light exposure and oxygen cause oxidative deterioration in butter, leading to losses of valuable nutrients and the formation of off-flavors.

The acid value of pasteurized butter samples had an average of 0.67±0.01 mg KOH/ml. Nearly similar acid values were reported by Koczon *et al*. (2008), Laikoja *et al.* (2017) and Méndez-Cid *et al*. (2017), but lower than Naik *et al*. (2020) while higher than El-Mossalami and Abdel-Hakeim (2014) and El-Safty *et al.* (2017). Moreover, the average acid value of Falahy butter samples was 0.82±0.02 mg KOH/ml which was like that recorded by Simsek (2011) and lower than the results of Naik *et al*. (2020) and El-Mossalami and Abdel-Hakeim (2014) while higher than the result of Ramadan *et al*. (2009). There was a statistically significant difference between acid values of pasteurized and Falahy butter (Table 2). The loss of stability of milk lipase is derived mainly from oxidative changes, since reducing agents greatly prolong the storage stability of this enzyme system. Milk lipase is very sensitive to photo-inactivation and ~~to~~ oxidizing agents. The stability of milk lipase to heat, light, hydrogen peroxide, and copper, like storage stability, is greater in whole milk than in skim milk (Frankel and Tarassuk, 1959). Acid value varies depending on the type and species of animal used to produce milk, butter composition, production parameters, storage conditions, and time. The enzymatic breakdown of milk lipids by lipase enzymes produces free fatty acids. Lipase comes from two sources: milk lipase, which is eliminated by pasteurization, and lipase produced by microbes (Ayar *et al*., 2006).

The microflora of butter reflects the quality of the cream, the sanitary conditions of the equipment used in butter production, and the

environmental and sanitary conditions during packing and handling (Meshref, 2010).

As seen from Table 3, first, in between each group of samples there was a big difference in the total bacterial count, second, bacterial count always was lower in Pasteurized butter which contained a min. of $1x10^2$ and a max. of $8x10^5$ cfu/g with a mean value of $1.3x10^5$. Otherwise in Falahy butter the min. count was $1.5x10^3$ and the max. count was $4.8 x10^6$ cfu/g with a mean count of $8.8x10^6$. These results are in harmony with those obtained by Osman *et al*. (2010), El-Derwy *et al*. (2011) and Ahmed *et al*. (2018) but lower than Rady and Badr (2003). The discrepancy between the TBC of Pasteurized and Falahy butter in these studies could be due to the difference between the method of handling, collecting of milk, manufacturing processes, and storage conditions which can affect the bacteriological quality of the product.

The same trend in the total bacterial count was obtained concerning counts of Psychrotrophic as presented in Table (3). The corresponding values of Psychrotrophic count in pasteurized butter were a min. of $1x10^2$ and max. count of $9 x10^5$ cfu /g and with a mean value of 1x105. On the other hand, values for Falahy butter samples were with a min. count of $5x10^2$ and a max. of $9.5 x 10^5$ cfu /g with a mean count of $3.2x10^5$. These results are higher than the results of those reported by Kasana *et al*., (2002), Kacem and Karam (2006), Idoui *et al*. (2010), Meshref (2010) and El-Derwy *et al*. (2011). Storage of such products for long period at refrigerated temperatures has resulted in quality problems usually by lipolytic and proteolytic activity produced during the growth of psychrotrophic which are heat stable and cause various off-tastes; like bitter, fruity, sour, putrid and unclean flavor. Also, clot formation, rancid, and in some cases, virtually complete digestion of protein could happen (Salama and Enan, 2005).

Additionally, the count of lipolytic bacteria in two groups of samples had the same trend as that obtained for the total and psychrotrophic bacterial count. The contamination rate of the lipolytic bacterial count was very high and closely in agreement 90, and 92.5%, with min. was $1 x10^2$ and $2 x10^2$ in Pasteurized and Falahy butter respectively. Max. Counts were $7x10^5$ and $9 x 10^5$ cfu/g with mean values $5.7 x10^4 \pm 1.3 x10^4$,$9.4 x10^4 \pm 2 x10^4$cfu/g for Pasteurized and Falahy butter samples, respectively. These results were higher than those found by Asresie *et al*. (2013), Idou *et al*. (2013), Ahmed *et al.* (2016) and Findik and Andik (2017) but lower than the results of El-maghraby *et al*. (2015).

The relatively high lipolytic count detected in the examined butter samples in this study may reflect the unhygienic sanitary precautions applied during their production, handling, and distribution as well as storage condition. Which have lipolytic activity responsible for the appearance of rancid smell in butter, and the rancidity is related to the appearance of compounds of unpleasant odors (acids, aldehydes, ketones) resulting from the hydrolysis of fat content by microbial lipases (Idou *et al*., 2013).

The yeast and mold were present in 78.75, 82.5 % in pasteurized and Falahy butter samples for yeast, 35 % in pasteurized, and 46.25% in Falahy butter samples for mold. The total numbers of yeast varied from $1x10^2$ to $1.4 x10^5$ in pasteurized butter with the mean value of $1.5x10^4 \pm 3 x10^3$ cfu/g. While in

Falahy butter samples total yeast count ranged from $1x10^2$ to $1.9x10^5$ with the mean value of $2.8\ x10^4 \pm 4.5x\ 10^3$. Also, mold count in both pasteurized and Falahy samples had a minimum value of $1x10^2$, $2x10^2$ cfu /g, while the maximum counts were $3\ x\ 10^4$, $2\ x10^5$ respectively. Moreover, the mean counts of total molds were $1.3\ x10^4 \pm 3.3\ x10^2$ in pasteurized butter samples and $1.6\ x10^4 \pm 4.2\ x10^3$ in Falahy butter samples. Similar counts were reported by Samet-Bali *et al*. (2009), Gökce *et al*. (2010), Idoui *et al*. (2010), Meshref (2010), Ahmed *et al*. (2016) and Gazu *et al*. (2018).

High moisture content in Falahy butter is not justified because of the preparation method. Unlike industrial preparation protocol, milk's fat does not remove completely, and water is substituted as a fat replacer in butter formulation (Idoui *et al*., 2013). Because the fat in milk is not completely removed in the industrial preparation technique, water is used as a fat substitute in butter manufacturing. So, the environment of the producing site is the main source of molds and yeasts entering butter samples, the presence of these bacteria is warranted, especially in traditionally manufactured butter (Incheh *et al*., 2017).

Factors influencing mold and yeast growth include sanitation during manufacturing and ripening, ripening length and degree, storage circumstances (temperature, relative humidity, type and extent of packing), water activity, and product composition. The high counts of yeast and molds found in this study's butter samples could be attributable to the butter samples' favorable growth circumstances during processing and cold storage (Wasswa *et al*., 2017).

CONCLUSIONS

This study revealed that the organoleptic and chemical properties of pasteurized butter samples were better than those of Falahy ones. Falahy butter samples showed a higher microbial load than pasteurized butter samples based on the results of TBC, Psychrotrophic bacterial count, lipolytic bacterial counts, yeast, and mold count. There were significant differences in the microbiological quality between pasteurized butter samples and Falahy ones.

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تقييم الجودة الكيميائية والميكروبيولوجية للزبد المباع في مدينة اسيوط – مصر

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تعتبر عملية أكسدة الدهن من أهم المشاكل التي تواجه كلا من المنتج والمستهلك للزبد نظرا لما ينتج عنها من تغيرات فيزيائية وكيميائية غير مرغوبة مثل انخفاض الجودة الحسية وكذلك القيمة الغذائية للمنتج. تتعدد العوامل التي تؤثر على جودة الزبد مثل ظروف التخزين من حيث درجة الحرارة والمدة، حموضة القشدة المستخدمة في الصناعة ونوع دهن اللبن المستخدم. تهدف هذه الدراسة إلى التقييم الصحي وجودة 160 عينة من الزبد المغلف والزبد الفلاحي (80 عينة لكل نوع) مجمعة عشوائيا من مدينة أسيوط - مصر. فقد خضعت العينات للفحص الحسي والفحص الميكروبيولوجي والتحليل الكيميائي (قياس رقم البيروكسيد، حمض الثيوباربيتيوريك ورقم الحموضة). وقد كشف الفحص الحسي أن ٧٨،٥% من عينات الزبد المغلف تُصنف كممتاز و٢١،٢٥% كجيد جدا أما بالنسبة للزبد الفلاحي فان ٦٢،٥% تصنف كممتازو٣٧،٥% تصنف كجيد جدا. أيضا كشفت نتائج التحليل الكيميائي أن المتوسطات العامة لكل من رقم الاكسدة وحمض الثيوباربيتيوريك، ورقم الحموضة لكل من الزبد المغلف والفلاحي كانت (٠،٣٩ - ٠،٤٤ مل معادل للأكسجين/كجم)، (٠،٤٦ - ٠،٥١ مجم مالونالدهيد /كجم)، (٠،٦٧-٠،٨٢ معادل بوتاسيوم هيدروكسيد /جم) على التوالي. وأسفرت نتائج الفحص الميكروبيولوجي عن أن المتوسطات العامة للعد الكلي البكتيري، البكتريا المقاومة للبرودة، الميكروبات المحللة للدهن، الخمائر والفطريات كانت ($10^5\times1,3\pm10^4\times1,6$, $10^6\times8,8\pm10^4\times1,3$)؛ ($10^5\times1\pm10^4\times2.3$, $10^5\times3.2\pm10^4\times3.3$) ؛ ($10^4\times2\pm10^4\times9.4$ ، $10^4\times1.3\pm10^4\times5.7$) ؛ ($10^4\times1.5\pm10^3\times3$, $10^4\times2.8\pm10^3\times4.5$) و ($10^3\times1.3\pm10^2\times3.3$, $10^4\times4.2\pm10^3\times1.6$) وحدة مستعمرة بكتيرية / جرام لكل من الزبد المغلف والفلاحي على التوالي. وتوصي هذه الدراسة بتشديد الرقابة الصحية على عملية تصنيع وتغليف وتخزين الزبد وخاصة الزبد الفلاحي، وكذلك نشر الوعي الصحي بهدف تقديم منتج صحي وآمن للمستهلك.