



## Antioxidant activity of raw milk and dairy products commonly consumed in Fars province, Iran

Maryam Jafari<sup>a</sup>, Gholamreza Jahed Khaniki<sup>a</sup>, Mehdi Roshanzamir<sup>b</sup>, Parisa Sadighara<sup>a\*</sup>

<sup>a</sup>Department of Food Safety and Hygiene, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

<sup>b</sup>Laboratory Manager, Pegah Fars Pasteurized Milk Co., Shiraz, Iran

### ARTICLE INFO

#### Article history:

Received 02 Apr. 2017

Received in revised form

11 Jul. 2017

Accepted 19 Jul. 2017

#### Keywords:

Antioxidant activity;

Dairy products;

Milk

### ABSTRACT

Dairy products play an important role in our daily diet. The objective of the present study was to assess the antioxidant capacity of raw milk and dairy products of Fars province, Iran. A total of 30 samples of raw milk and commonly consumed dairy products including high temperature short time (HTST) and ultra-high temperature (UHT) treated milk, hard cheese, doogh (drink yogurt) and yogurt were collected from a dairy plant in which raw milk bulk was supplied by farms located in Shiraz and Marvdasht regions of Fars province, Iran. The antioxidant capacity of the samples was determined using two spectrophotometric methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and cupric reducing antioxidant capacity (CUPRAC) assays. The results were expressed as mg of dry matter. The DPPH radical-scavenging activity of raw, sterilized and pasteurized milk samples were similar and ranged from  $8.46 \pm 0.04$  to  $8.75 \pm 0.08$  mM EVCAA /100g dw. However, these samples exhibited significant differences ( $P < 0.001$ ) among their reducing power capacities. Among the products, cheese represented the highest DPPH radical-scavenging capacity ( $5.02 \pm 0.01$  mM EVCAA /100g dw;  $p < 0.001$ ), while the highest reducing power activity was found in cheese samples ( $262.84 \pm 0.18$  mM EVCAA /100g dw;  $p < 0.001$ ). Dairy products offered a promising performance as the source of natural antioxidants.

**Citation:** Jafari M, Jahed Khaniki Gh, Roshanzamir M, Sadighara P. **Antioxidant activity of raw milk and dairy products commonly consumed in Fars province, Iran**, J Food Safe & Hyg 2017; 3(1-2): 21-26.

### 1. Introduction

Antioxidants are essential to reduce or prevent different types of cancer, cardiovascular and neurological diseases, arteriosclerosis and aging-related disorders. Although some synthetic antioxidants are commonly used in foods, the possible toxicity of such substances has attracted the attention of both the manufacturers and consumers to natural antioxidants. Therefore, there is a considerable interest in the proper assessment of the antioxidant capacity of foods (5,25). Beyond the presence of micro - and macronutrients, dairy products are considered as good

sources of such antioxidants which can be effective in preventing lipid peroxidation of these products and also in enhancing the oxidative defense of consumers (8,9,29). Enzymatic systems including superoxide dismutase, catalase and selenium-containing glutathione peroxidases, as well as the iron-binding protein lactoferrin, serum albumin, ascorbic acid, vitamin E (tocopherols and tocotrienols), some carotenoids and flavonoids and also amino acids, such as tyrosine and cysteine, are antioxidant components found in milk; the concentration of these substances, however, are influenced by cow feeding rations and

Corresponding author. Tel.: 00982188954914

\*E-mail address: [sadighara@tums.ac.ir](mailto:sadighara@tums.ac.ir)

milk storage conditions (7,14). In addition, heat treatment, fermentation and proteolysis have been shown to affect the antioxidant properties of milk (1,10,20). Concerning the milk antioxidant activity, available data are those obtained from studies carried out on the individual antioxidants and the scarce information is available about the total antioxidant capacity (TAC) of dairy products (8).

Various methods, based on different mechanisms, are used to assess the antioxidant activity of foods. In previous studies, the TAC of different dairy products including raw, HTST-pasteurized and UHT-sterilized milk, whey, kefir, yogurt, cheese and fermented milk have been evaluated using different spectrophotometric methods such as the Trolox equivalent antioxidant capacity (TEAC) assay, the ferric reducing ability (FRAP) assay, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay, the oxygen radical absorbance capacity (ORAC) assay and the modified copper reduction (CUPRAC) assay (2,9,12,23).

Since the antioxidant capacity of dairy products is rarely investigated in Iran, the primary aim of the current study was to assess and compare antioxidant activities of raw milk and commonly consumed dairy products including HTST-pasteurized and UHT-sterilized milk, cheese, doogh (Iranian drinking yogurt) and yogurt in Fars province, Iran by 2 different spectrophotometric procedures: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and cupric reducing antioxidant capacity (CUPRAC) assays. Fars province is one of the most important parts of the country contributing to the dairy industry in which the main industrial dairy herds are those of three main regions (Shiraz, Marvdasht, and Sepidan) (11). Of these dairy herds, those of Shiraz and Marvdasht regions supply the bulk raw milk used in Pegah dairy processing plant (Fars, Iran) from which the study population was collected.

## 2. Materials and methods

### 2.1. Chemicals and instruments

All chemicals and reagents were of analytical grade from Merck Co. (Darmstadt, Germany) and Sigma Aldrich (Steinheim, Germany). The spectrophotometer used was SPECORD 205 (Analytical Jena).

### 2.2. Sampling

During the summer of 2013, the antioxidant activity of 90 different samples collected from Pegah dairy

processing plant (Fars province, Iran) was evaluated. Cow's raw milk samples (3% fat) were taken from bulk storage tanks; HTST-pasteurized and UHT-sterilized milk (2.5% fat), hard cheese, doogh (drink yogurt) and yogurt samples were collected while coming off the production line.

### 2.3. Determination of total solids

Moisture was determined by the method of the Association of Official Analytical Chemists (AOAC, 1999). In brief, approximately 5 g of each homogenized sample was weighed and poured in separate dry aluminum dishes. The dishes were then placed in an air oven (Mettler, Schwabach, Germany) regulated to  $102 \pm 2$  °C; then, the samples were drained to constant weight (about 2h). Afterward, they were transferred to a desiccator to become cool and to be weighed. Weight loss was calculated as moisture content.

### 2.4. Sample preparation

Almost 20 mL of 90% methanol was added to 5 g of each sample. Following centrifugation at 4000 rpm, 10 min at 20 °C; the supernatant was collected, filtered through Whatman No.1 filter paper and then stored at 4°C.

### 2.5. Antioxidant activity tests

#### 2.5.1. Scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical

The DPPH radical-scavenging capacity of samples was determined according to the modified method of (Marinova et al. 2011). Two concentration of extract samples were used. Briefly, 1ml of extract samples were added to 1 ml of DPPH reagent, and an aliquot of extract (250  $\mu$ L) to 2,250 ml methanol was mixed with 1 mL of freshly prepared DPPH reagent (Dilute 0.0024 g DPPH in 100 ml ethanol (0.06 mM). Attemperate 20 min to 20o C. Prepare fresh every day). The reaction solution was then shaken and incubated at room temperature in the darkness for 30 min. The absorbance of the resulting solution was measured against a reagent blank at 517 nm.

#### 2.5.2. Assessment of cupric ion reducing antioxidant capacity (CUPRAC)

CUPRAC was measured according to the method described by (3) with some modifications. The reaction mixture containing 1 mL of copper (II) chloride solution

(0.01 M), 1 mL of neocuproine (2, 9-Dimethyl-1, 10-phenanthroline) alcoholic solution (0.0075 M in ethanol), 1 mL of ammonium acetate aqueous buffer (19.27 g in 250 mL of water, pH 7.0) and 1 mL of extract was incubated at room temperature for 30 minutes, and the absorbance was measured against a reagent blank at 450 nm.

### 2.5.3. Calibration curves

For these two methods, ascorbic acid was used as a standard and the standard solutions were prepared by methanol and ethanol.

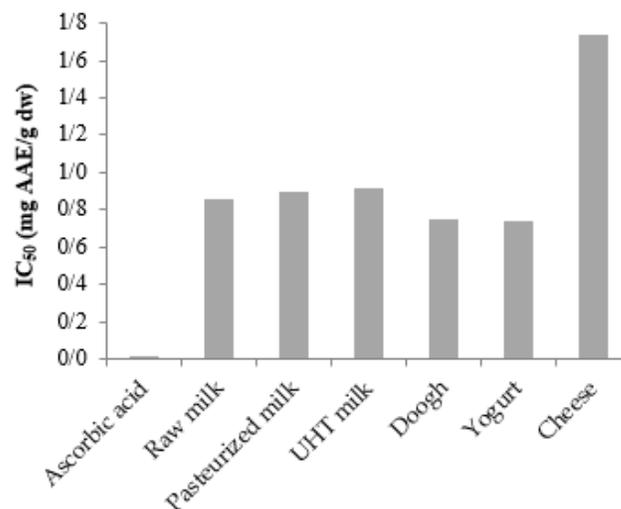
### 2.6. Statistical analysis

Statistical analysis (ANOVA with a statistical significance level set at a confidence interval of 95% ( $P < 0.05$ ) and linear regression), was used to calculate Median Inhibition Concentration (IC<sub>50</sub>) value, calibration curves drawing for DPPH; also, correlation analysis of total antioxidant capacity was carried out using Microsoft Excel 2010. Each sample analysis was performed in triplicate. All results presented are means ( $\pm$ SD) of at least three independent experiments.

### 3. Results

As previously mentioned, consumption of foods containing physiologically active components such as antioxidants is considered to be a promising solution for the prevention of degenerative diseases; Dairy products are undoubtedly included as important components of a balanced diet (19) which could be considered as the sources of natural antioxidants, as well. Thus, the antioxidant activity of raw, HTST-pasteurized and UHT-sterilized milk, doogh (drink yoghurt), yoghurt and cheese samples produced in a

dairy plant was investigated. The results obtained by two different methods are represented in Table 1.



**Figure 1.** IC<sub>50</sub> values (concentration that reduces the effect by 50%) of different dairy product samples in comparison to that of Ascorbic acid (AA). Values are given as mean value of three replicates. Different capital letters indicate significant differences ( $P < 0.05$ ) between samples (vertical bars indicate SD of the mean). AAE: ascorbic acid equivalent; dw: dry weight.

### 4. Discussion

As various methods and units have been used to evaluate and report the antioxidant capacity of dairy products in previous studies, a direct comparison of the results was difficult. On the other hand, the methods giving the most reliable results for some food matrices may not be suitable for other foods. In the present study, the antioxidant activity was evaluated using two different methods (DPPH and CUPRAC methods). Despite the fact that the CUPRAC method is capable of assessing the antioxidant activities of both hydrophilic and lipophilic compounds and also the antioxidants

**Table 1.** Evaluation of the antioxidant capacities of different milk types by two methods: DPPH (mg EVCAA /g dw) / (mM EVCAA /g dw) and CUPRAC (mg EVCAA /g dw) / (mM EVCAA /g dw).

Sample	Number of samples	Total solids (%)	Antioxidant activity	
			DPPH- scavenging activity (mg/g)	CUPRAC ( $\mu$ g/g)
Raw milk	5	9.93 $\pm$ 0.02	Bc 8.75 $\pm$ 0.08	Aa 0.04 $\pm$ 0.01
HTST Pasteurized milk	5	10.32 $\pm$ 0.05	Bc 8.73 $\pm$ 0.08	Bb 0.94 $\pm$ 0.05
UHT sterilized milk	5	10.89 $\pm$ 0.04	Bb 8.46 $\pm$ 0.04	Dc 5.47 $\pm$ 0.05
Doogh	5	7.35 $\pm$ 0.025	Cd 10.25 $\pm$ 0.03	Cb 1.19 $\pm$ 0.02
Yoghurt	5	12.14 $\pm$ 0.05	Aa 6.13 $\pm$ 0.04	Cb 1.71 $\pm$ 0.01
Cheese	5	34.75 $\pm$ 0.036	Aa 5.02 $\pm$ 0.01	Ee 262.84 $\pm$ 0.18

Values are given as mean  $\pm$  SD of three replicates. Different lowercase letters in line indicate significant differences ( $P < 0.05$ ) among assessment method for each sample, whereas different capital letters in column indicate significant differences ( $P < 0.05$ ) between samples analysed using the same method. DPPH: 2, 2-diphenyl-1-picrylhydrazyl; CUPRAC: cupric ion reducing antioxidant capacity; dw: dry weight; EVCAA (equivalent vitamin C antioxidant activity)

detected by DPPH method are limited to those soluble in organic solvents (especially alcohols) (8), DPPH method yielded much higher values compared with the CUPRAC procedure and there was a slight correlation ( $r^2 = 0.88$  -1) between the DPPH and CUPRAC values obtained for the various samples. In the study conducted on the antioxidant activity of raspberry and blackberry, the CUPRAC assay exhibited lower antioxidant activity values than DPPH assay; however, they showed high positive correlation (21). Both DPPH and CUPRAC methods share a single electron transfer principle. In the DPPH assay, the capacity of the sample in scavenging of DPPH radicals is evaluated and the decolorization of the purple DPPH radical to yellow is an indication of the scavenging activity of the antioxidant (decrease absorption = increase antioxidant activity) (24). In the CUPRAC method, cupric ion (Cu (II) to Cu (I)) reducing ability is used as a criterion on the antioxidant capacity of the sample (increase absorption = increase antioxidant activity) (8). However, it has been demonstrated that the antioxidant capacity measured by FRAP or CUPRAC method does not always correlate well with that for radical scavenging (17). Although CUPRAC method has been recommended to evaluate the phenolic antioxidants of plant foods (3) and among all the assays used by some researchers (25) it has been suggested to determine the total antioxidant activities of several beverages, DPPH has been shown as the least sensitive; it seems that DPPH assay is more sensitive than the CUPRAC method for measurement of antioxidant capacity of dairy products. However, as reported by Çekiç et al. (2009), the standard CUPRAC method modified by substituting the ammonium acetate buffer with urea buffer is expected to be useful in estimating the total antioxidant capacity of dairy products and other protein-containing foods. In general, various methods employed to evaluate the antioxidant capacity of a specific product can yield different results; this is in agreement with those reported by Tabart et al. (2009). In this study, the DPPH-scavenging capacity of raw milk was comparable to that of heat-treated milk samples, however, it showed a significantly lower cupric reducing ability when compared to HTST ( $P < 0.001$ ) and UHT treated milk samples ( $P < 0.001$ ). Such observations are not unexpected, because CUPRAC method is capable to simultaneously measure lipophilic and hydrophilic antioxidants and in DPPH method only lipophilic ones are measured (8); thus while different milk samples with similar fat contents did not show different DPPH values, significant differences were found among their respective

CUPRAC values. In addition, although thermal treatments are considered to increase the milk antioxidant activity, previous studies (7,9) have demonstrated that only the application of severe heat treatments, associated with the formation of the Maillard reaction products, could affect the antioxidant activity of milk and also the antioxidant properties of milk are not influenced by mild heating i.e. below 100 °C and at relatively short times ( $\leq 1$  min). In the study conducted by Zulueta et al. (2009), the total antioxidant capacities of UHT and pasteurized milk were not significantly different. It is noteworthy that under the time-temperature combinations, commonly applied at the industrial level, for HTST-pasteurized and UHT-sterilized milk, only the early phase of the Maillard reaction takes place which leads to the formation of highly reactive radicals; thus, depletion of the antioxidant capacity of such milk products can occur (7). This is consistent with the data presented in this paper showing no significant differences between different milk samples while the Maximum activities were recorded for UHT-milk samples. Furthermore, the significant differences observed among these samples when analyzed using CUPRAC assay were very small in practical terms and could be attributed to the production of free H<sub>2</sub>S during heating processes which specifically removes CU<sup>2+</sup> ions (28).

Milk fermentation with proteolytic starter cultures and also enzymatic proteolysis that occurs during cheese manufacturing can lead to release antioxidative peptides from caseins and such bioactive peptides have been isolated from many dairy products including cheese, kefir and yoghurt (1,2,10,22). Milk and soymilk fermented by kefir grains have been reported to have greater DPPH-scavenging activity and ferric reducing power than milk and soymilk (15). Previous research reported that fermentation with *Lactobacillus casei* strain Shirota enhanced the DPPH radical-scavenging activity of skim milk (18). In the study conducted by Virtanen et al. (2007), whey fractions obtained from milk fermented with different strains of lactic acid bacteria exhibited the radical scavenging activity with inhibition rate in the range of 3–53% which was lower than that of the control milk that showed 100% inhibition. In another study, skim milk samples showed higher 2,2'-azino-bis-(3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium (ABTS) radical scavenging ability than fermented milk samples collected from the local stores (12). In fact, the extent of the antioxidant activity of fermented products is proportionally dependent on the type of the starter culture used (27). In the present study, cheese higher

than Doogh and yogurt showed DPPH-scavenging activity; however, the former showed higher values. An explanation for this difference is that this product is produced with different starter cultures. On the other hand, the reducing power of doogh and yoghurt samples was similar and greater than that of the raw milk. The reducing activity of lactic acid bacteria (*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Bifidobacterium longum*) has been reported in previous studies (13). According to the study conducted by Morandi et al. (2011), *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, the species used in yogurt and doogh production, have low reduction power (16). The highest reducing power was observed in cheese samples which may have resulted from the low oxidation-reduction potential (Eh) of cheese. The Eh of milk is about +150 mV whereas that of cheese is about -250 mV. Although the exact mechanisms by which the Eh of cheese is decreased is not well established, it is probably due to the fermentation of lactose to lactic acid by the starter culture and also the partial reduction of O<sub>2</sub> in milk to H<sub>2</sub>O (6,26).

The measured IC<sub>50</sub> was highest in cheese and lowest in doogh and yoghurt (Fig. 1). A lower IC<sub>50</sub> value indicates a lower DPPH free radical scavenging activity. It seems that extended fermentation time would result in a higher radical scavenging power of milk which is in agreement with the results obtained by some other researchers (15).

Finally, it should be noted that different samples analyzed in this study were not obtained from the same batch of raw milk and, thus, the effect of matrix variation on their respective antioxidant activity is not avoidable.

## 5. Conclusions

All the studied samples exhibit acceptable antioxidant capacity. Furthermore, commercial heat treatments do not substantially influence the milk antioxidant activity; while some technological treatments such as fermentation, concentration and ripening seemed to exert some positive influences on the antioxidant capacity of raw milk. Thus, beyond supplying the valuable macro- and micro-nutrients, dairy products offer a promising performance as the source of natural antioxidants which confer health-promoting features.

## Conflict of interest

The authors have no conflict of interest.

## Acknowledgements

The authors would like to thank the Center for Development of Clinical Research of Nemazee Hospital and Dr. Nasrin Shokrpour for editorial assistance.

## References

1. Aloglu HS, Öner Z. Determination of antioxidant activity of bioactive peptide fractions obtained from yogurt. *J Dairy Sci* 2011; 94: 5305–5314.
2. Alsayadi M, Al jawfi Y, Belarbi M, et al. Antioxidant potency of water kefir. *J Microbiol Biotechnol Food Sci* 2013; 2: 2444-2447.
3. Apak R, Güçlü K, Demirata B, et al. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecul* 2007; 12: 1496-1547.
4. Association of Official Analytical Chemists (AOAC), 1999. *Official Methods of Analysis*. 16th Ed, 5th Rev.
5. Bandyopadhyay M, Chakraborty R, Ray U. A process for preparing a natural antioxidant enriched dairy product (Sandesh). *LWT* 2007; 40: 842-851.
6. Beresford TP, Fitzsimons NA, Brennan NL, et al. Recent advances in cheese microbiology. *Int Dairy J* 2001; 11: 259-274.
7. Calligaris S, Manzocco L, Anese M, et al. Effect of heat-treatment on the antioxidant and pro-oxidant activity of milk. *Int Dairy J* 2004; 14: 421-427.
8. Çekiç SD, Başkan KS, Tütem E, et al. Modified cupric reducing antioxidant capacity (CUPRAC) assay for measuring the antioxidant capacities of thiol-containing proteins in admixture with polyphenols. *Talanta* 2009; 79: 344-351.
9. Chen J, Lindmark-Måansson H, Gorton L, et al. Antioxidant capacity of bovine milk as assayed by spectrophotometric and amperometric methods. *Int Dairy J* 2003; 13: 927-935.
10. Gupta A, Mann B, Kumar R, et al. Antioxidant activity of Cheddar cheeses at different stages of ripening. *Int J Dairy Technol* 2009; 62(3): 339-347.
11. Haghkhah M, Ansari-Lari M, Novin-Baheran AM, et al. Herd-level prevalence of *Mycobacterium avium* subspecies *paratuberculosis* by bulk-tank milk PCR in Fars province (southern Iran) dairy herds. *Prevent Vet Med* 2008; 86: 8-13.
12. Jiménez AM, Murcia MA, Parras P, et al. On the importance of adequately choosing the ingredients of yoghurt and enriched milk for their antioxidant activity. *Int J Food Sci & Technol* 2008; 43: 1464-1473.
13. LinM Y, Yen CL. Antioxidative ability of lactic acid bacteria. *J Agri Food Chem* 1999; 47: 1460-1466.
14. Lindmark-Månsson H, Åkesson B. Antioxidative factors in milk. *British J Nutr* 2000; 84: S103-S110.
15. Liu JR, Chen MJ, Lin CW. Antimutagenic and antioxidant properties of milk-kefir and soymilk-kefir. *J Agri Food Chem* 2005; 53: 2467-2474.

16. Morandi S, Brasca M, Lodi R. Technological, phenotypic and genotypic characterisation of wild lactic acid bacteria involved in the production of Bitto PDO Italian cheese. *Dairy Sci & Technol* 2011; 91: 341-359.
17. Niki E. Antioxidant capacity: Which capacity and how to assess it? *J Berry Res* 2011; 1: 169-176.
18. Nishino TH SS, Kikuchi-Hayakawa H, Ishikawa F. Transit of radical scavenging activity of milk products prepared by Maillard reaction and *Lactobacillus casei* strain Shirota fermentation through the hamster intestine. *J Dairy Sci* 2000; 83: 915-922.
19. Pereira PC. Milk nutritional composition and its role in human health. *Nutr* 2014; 30: 619-627.
20. Pritchard SR, Phillips M, Kailasapathy K. Identification of bioactive peptides in commercial Cheddar cheese. *Food Res Int* 2010; 43: 1545-1548.
21. Sariburun E, Şahin S, Demir C, et al. Phenolic content and antioxidant activity of raspberry and blackberry cultivars. *J Food Sci* 2010; 75(4): 328-335.
22. Songisepp E, Kullisaar T, Hütt P, et al. A new probiotic cheese with antioxidative and antimicrobial activity. *J Dairy Sci* 2004; 87: 2017-2023.
23. Sreeramulu D, Manchala R. Antioxidant and phenolic content of nuts, oil seeds, milk and milk products commonly consumed in India. *Food Nutr Sci* 2011; 2: 422-427.
24. Suetsuna K, Ukeda H, Ochi H. Isolation and characterization of free radical scavenging activities peptides derived from casein. *J Nutr Biochem* 2000; 11: 128-131.
25. Tabart J, Kevers C, Pincemail J, et al. Comparative antioxidant capacities of phenolic compounds measured by various tests. *Food Chem* 2009; 113: 1226-1233.
26. Topcu A, Mckinnon I, Mcsweeney PLH. Measurement of the oxidation-reduction potential of cheddar cheese. *J Food Sci* 2008; 73(3): 198-203.
27. Virtanen TA P, Akkanen S, Korhon H. Development of antioxidant activity in milk whey during fermentation with lactic acid bacteria. *J Appl Microbiol* 2007; 102: 106-115.
28. Walstra P, Geurts TJ, Noomen A, et al. *Dairy technology: principles of milk properties and processes*. New York: Marcel Dekker, Inc., 1999.
29. Zulueta A, Maurizi A, Frígola A, et al. Antioxidant capacity of cow milk, whey and deproteinized milk. *Int Dairy J* 2009; 19: 380-385.
30. Marinova G, Batchvarov V. Evaluation of the methods for determination of the free radical scavenging activity by DPPH. *Bulgar J Agri Sci Agri Acad* 2011; 17: 11-24.