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Copper, iron and zinc variations in Manchego-type cheese during the traditional cheese-making process

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Variations in mineral content were determined throughout the process of making cheese by taking samples of natural, pasteurized milk, with additions of rennet, curd, whey, pressed curd, pressing whey, and cheese. The mean contents of copper, iron, and zinc in cheese were 1·03, 6·71, and 37·86 μg/g, respectively, in fresh weight. The contribution of the consumption of this type of product to the daily-intake estimates in a Spanish diet are 3·22 μg/day for copper, 20·9 μg/day for iron, and 117·8 μg/day for zinc. By an analysis of variance, the existence of statistically significant differences ($p < 0{\cdot}001$) was confirmed in the products from the cheese-making for the three minerals, expressed as both fresh weight and dry weight. Certain differences were observed in the groups formed on using a Scheffe homogeneity test ($p < 0{\cdot}05$) depending on whether the mineral content was expressed as fresh weight or as dry weight. Slight rises in the contents of the three minerals investigated as dry weight were attributed mainly to the retention time of the minerals by the curd and secondly, to possible contamination occurring during the process.

Key words: Minerals, Cheese-making, ASS

INTRODUCTION

Manchego cheese is the most popular indigenous Spanish cheese, consumed both in Spain and outside its borders. Its average yearly production is estimated to be over 50 000 tonne/year (Fox, 1987). This product is made by following a traditional process using different proportions of ewe's, goat's, and/or cow's milk, and there are also many other cheeses which, although they do not receive the denomination of Manchego, are made in the same way.

The mineral content of the cheese is very variable and depends on several factors, such as (*a*) the differences in mineral content among the species producing the milk from which it is made (Franco *et al.*, 1981; Garcia Olmedo *et al.*, 1981*a,b*); (*b*) the geographical area in which it is made (Coppini *et al.*, 1979); (*c*) the characteristics of the cheese-making process (Feeley *et al.*, 1972); (*d*); the degree of ripening (Coppini *et al.*, 1979); and (*e*) the conditions under which this ripening is carried out (Le Graet & Brulé, 1988).

The literature consulted indicated very variable concentrations in the contents of these trace elements in different cheeses. Coppini *et al.* (1979), in six-months-ripe Parmesan cheese, found concentrations in dry matter of copper of between 8·6 and 10·6 mg/kg; of iron between 10·5 and 17·9 mg/kg; and of zinc between 35·5 and 40·2 mg/kg. Gabrielli Favretto (1990) indicated concentrations in cheese of 0·54, 1·76, and 34·6 mg/kg for copper, iron, and zinc, respectively. Gabrielli Favretto and Pertoldi Marletta (1984) observed concentrations of 0·31 mg/kg for copper and 2·32 mg/kg for iron in cheese. Koops *et al.* (1986) found a content of zinc of 39·1 mg/kg in four-month-ripe Gouda cheese. Le Graet and Brulé (1988) determined concentrations of 0·5 mg/kg of copper, between 0·63 and 1·48 mg/kg of iron, and between 24·6 and 35·8 mg/kg of zinc in Camembert-type cheese of five days. The concentrations of copper and iron obtained by Pertoldi Marletta and Gabrielli Favretto (1983) in cheese were 0·43 and 3·25 mg/kg, respectively. Wong *et al.* (1978) reported variability in the concentrations of iron in cheese of between 1·45 and 9·5 mg/kg.

The aim of this study is to determine the influence of the traditional process of making Manchego-type cheese on its mineral content. This could be determined by two main factors: the relation of each one of the minerals studied to the curdling and, soluble fractions of the milk (Renner, 1989) and the possible contamina-

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tion that is introduced in the process (Pertoldi Marletta & Gabrielli Favretto, 1983).

MATERIALS AND METHODS

A routine production batch of Manchego-type cheese made in a commercial cheese-making factory in Spain was studied.

A mixture of milk (22% goat's milk and 78% cow's milk) was pasteurized and subsequently placed in the curdling vat, where 3350 litres of milk with a 3·9% fat content and 15·5° Dornic acidity was processed. Addition to the milk of 70 g ferment (Bioferment Dac Homo), 280 g discolouring agent, 175 cm^3 lysosyme, 670 g $CaCl_2$, and 800 cm^3 rennet (Proinalsa) was made. After the formation of curds, these were pressed in plastics moulds. The pressed curd was placed in brine with a 20% salt content, pH 5·3 and 13° Dornic, in which it remained for 40 hours.

During the process, ten statistically random samples were taken of each one of the following stages of the process: natural milk, pasteurized milk, the addition of ferment, curd, whey exuded from the curd, pressed curd, whey from pressed curd, and fresh cheese.

For the mineralization of the samples, the work of Gabrielli Favretto (1990) was followed. The mass of the sample used for analysis in each case depended on its nature; for liquid samples, 50 g were taken and, for the solid ones, 10 g.

The crucibles containing the dried samples were incinerated in a furnace at 460°C overnight. After cooling, 2 N nitric acid (2 ml) was added, and the solutions were dried on a thermostatic hotplate. They were subsequently placed once again in the furnace, where they remained at 460°C for one hour. The recovery of the ash was carried out with 2 N nitric acid (5 ml) and 0·1 N nitric acid (20 ml) in a 25-ml volumetric flask (subsequently stored in polypropylene flasks under refrigeration).

The determinations were performed with a Perkin–Elmer Model 2380 Atomic Absorption Spectrophotometer. A 10-cm one-slot burner head and standard air—acetylene flame and wavelengths of 324·8 nm, 248·3 nm, and 213·9 nm, for copper, iron, and zinc, respectively, were used. Simple-element hollow-cathode lamps were used for all elements. The instrument settings and other experimental conditions were in accordance with the manufacturer's specifications. The sensitivity obtained was 0·045 mg/litre; 0·052 mg/litre and 0·440 mg/litre for copper, iron, and zinc, respectively. The mean spiked recoveries were copper = 92%, iron = 101%, and zinc = 95%; Table 1 shows the mean recoveries from certificate standard NBS 1549 (Non-fat Milk Powder). For the calculation of the detection limit, the criteria of the American Chemical Society (1980) and Mottola (1984) were followed. The concentration limits obtained (minimum concentrations detectable in fresh weight) were 0·015 mg/kg, 0·053 mg/kg, and 0·675 mg/kg for copper, iron, and zinc, respectively.

Table 1. Mean recoveries (mg/kg) from certificate standard NBS 1549 (Non-fat Milk Powder)

Element	NBS 1549	Found
Cu	0·70 ± 0·1	0·68 ± 0·04
Fe	1·78 ± 0·1	1·80 ± 0·07
Zn	46·1 ± 2·2	46·2 ± 0·80

Statistical analysis

Data obtained from the chemical analysis of the samples were evaluated statistically by using a two-factor variance analysis with the Scheffe multiple-range test (Snedecor & Cochran, 1971).

RESULTS AND DISCUSSION

Table 2 shows the mean concentrations of copper, iron, and zinc and Figures 1, 2, and 3 their evolutions at fresh (A) and dry (B) weight through the eight products analysed.

A one-factor analysis of variance was used to determine if there were significant differences between the products studied, and it was found that there were differences both in the moisture content and in the contents of the three elements investigated (expressed at both fresh and dry weights) ($p < 0·001$) after which a

Table 2. Moisture content (%) and contents of copper, iron, and zinc (mean ± sd) expressed as mg/kg (at fresh weight) in the different products formed in the cheese-making process: homogeneous group from Scheffè multiple-range test (p < 0·05) as fresh and dry weights

Product	Moisture (%)	Copper	Iron	Zinc
Natural milk	89·2 ± 0·1^{D}	0·185 ± 0·020*A/a*	0·530 ± 0·035*A/a*	3·585 ± 0·085*A/b*
Pasteurized milk	89·3 ± 0·1^{D}	0·176 ± 0·007*A/a*	0·574 ± 0·034*A/a*	3·590 ± 0·165*A/b*
Milk + rennet	89·2 ± 0·1^{D}	0·171 ± 0·030*A/a*	0·595 ± 0·071*A/a*	3·575 ± 0·109*A/b*
Curd	54·7 ± 0·4^{C}	0·901 ± 0·176*B/ab*	2·945 ± 0·257*B/a*	27·675 ± 1·054*B/c*
Curdling whey	94·2 ± 0·2^{E}	0·094 ± 0·012*A/a*	0·294 ± 0·050*A/a*	0·200 ± 0·041*A/a*
Pressed curd	47·8 ± 0·2^{A}	1·245 ± 0·450*C/c*	2·985 ± 0·509*B/a*	31·492 ± 1·362*B/c*
Pressing whey	95·0 ± 0·1^{F}	0·178 ± 0·024*A/d*	3·466 ± 0·454*B/c*	1·505 ± 0·086*A/b*
Cheese	49·2 ± 0·5^{B}	1·037 ± 0·216*B/b*	6·712 ± 6·166*C/b*	37·867 ± 12·689*C/d*

A,B,C,D,E,F Scheffè homogeneous groups ($p < 0·05$) at fresh weight.
a,b,c,d Scheffe homogeneous groups ($p < 0·05$) at dry weight.

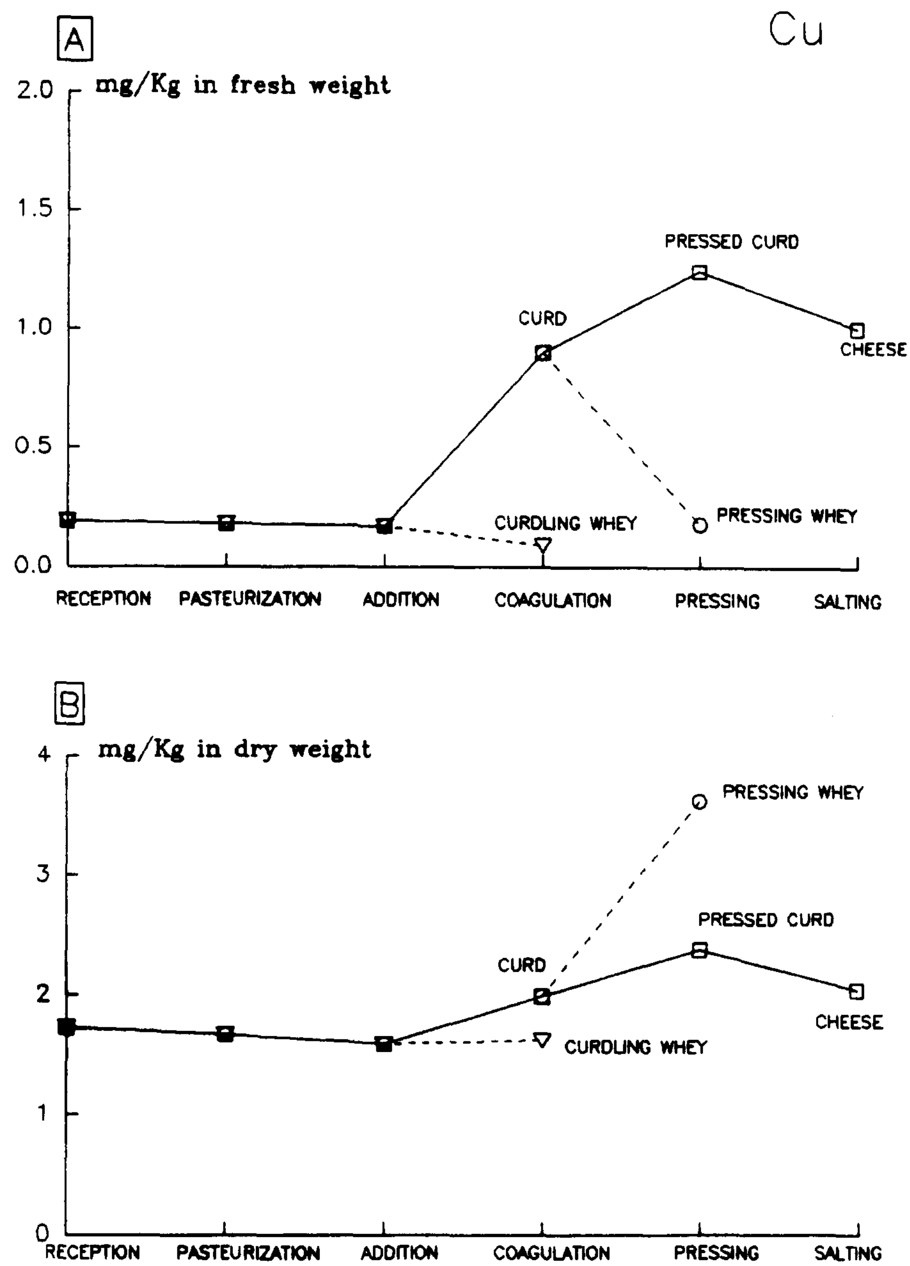

Fig. 1. Copper evolution during the cheese-making process.

Scheffe multiple-range analysis ($p < 0{\cdot}05$) was carried out and the groups observed in Table 2 were ascertained.

Each Scheffe homogeneous group is formed by a group of means that do not present any statistically significant differences from each other. Products marked with the same letter for an element or moisture content had similar concentrations.

In the homogeneous groups formed with the Scheffe test, it was observed that the moisture contents of the various resulting products were different except in the milks, which formed on group in themselves.

For copper and zinc at fresh weight, the liquid products formed a homogeneous group. In iron, however, the pressing whey showed an abnormally high content (from six to ten times as high as that of the other liquids), which meant that it did not form a group with the liquids but it did do so with the curds.

If we observe the concentration of the three elements in the pressing whey as compared with the curdling whey (the pressing whey differs from the latter only in the system used to facilitate its exit), it can be seen how the pressing whey shows higher concentrations than the curdling whey, and this is much more apparent at dry weight.

Furthermore, we observed some samples of pressed curd and cheese with high levels of copper, iron, and/or zinc. These abnormal concentrations in pressing whey and fresh cheese could be due to contamination by these elements in the manufacturing process (pressing and salting), a fact previously noted by Pertoldi Marletta & Gabrielli Favretto (1983) in the making of Parmesan cheese, when contamination by iron was observed.

In order to verify the hypothesis of a possible contamination, analyses were made on three portions

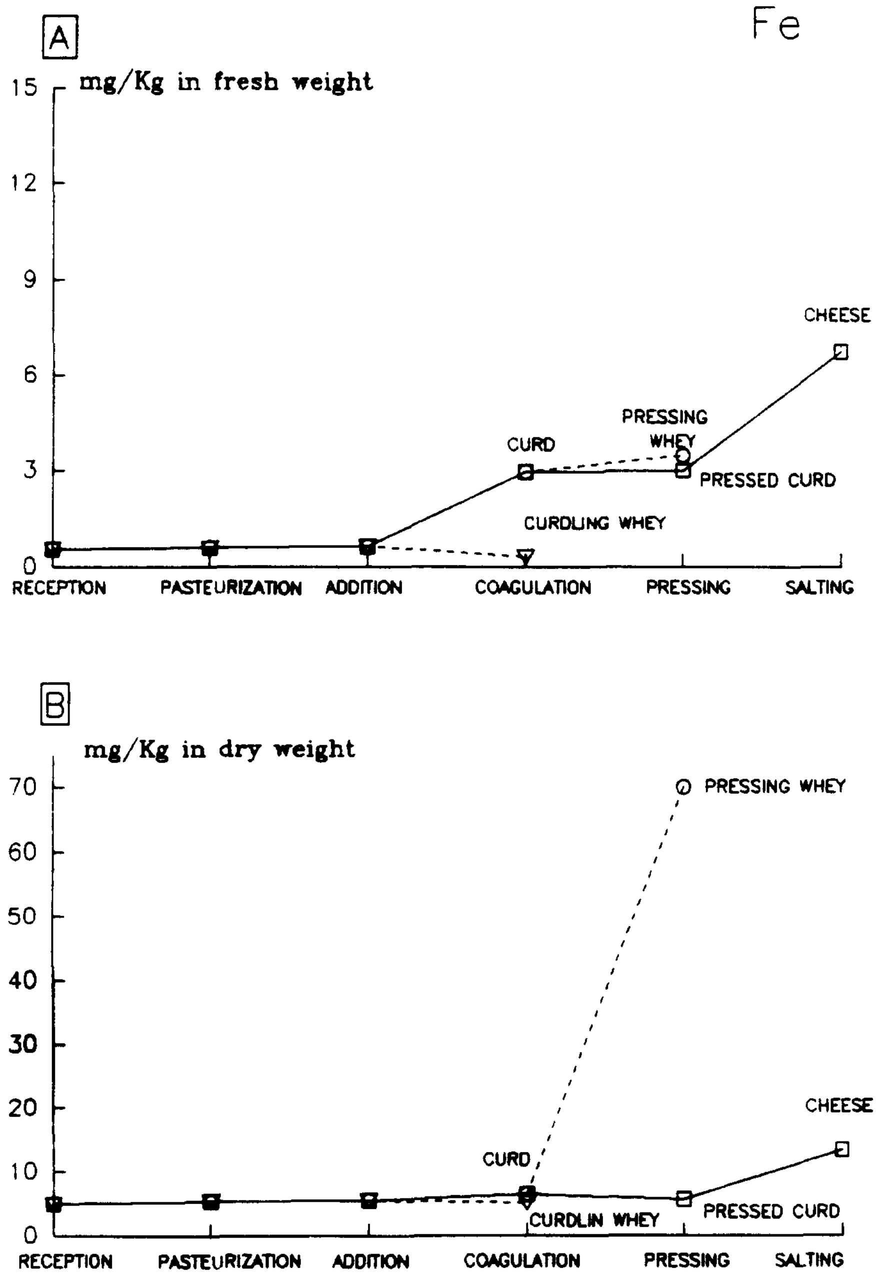

Fig. 2. Iron evolution during the cheese-making process.

situated at different depths, both in the pressed curd and in the cheese, and statistically significant differences ($p < 0{\cdot}001$) were found between the rind portions and other deeper ones, in iron and zinc, as both fresh and dry weights in the two products. For instance, in cheese, expressed as fresh weight, iron was present at 13·7 μg/g in the rind and 3·21 μg/g in the other portions and zinc was found at 55·0 μg/g in the rind and at 29·3 μg/g in the other portions, so that the rind portion was found to have higher values in all the samples analysed.

We believe that the use of metallic elements, such as iron weights and galvanized sheets, which come into contact with the pressing whey, which in turn impregnates the curds situated below it, could be the source of such contamination, which is more or less intense depending on the position of each cheese in the press.

In the case of copper, no clear preponderance of some portions over others was found, although, on studying individually the cheeses analysed, some statistically significant differences were observed between portions, and the preponderance of some portions over others was variable. This could be due to contamination prior to the pressing and, to be more precise, during curdling, since the cutter machine for curdling contains copper elements.

These types of contamination, although slightly increasing the concentrations of the three minerals investigated in the final products, may entail more of a technological risk than a nutritional benefit, since the minerals are free in the cheese and may therefore cause elevated catalytic oxidation of cheese lipids (Jimenez *et al.*, 1984).

If the sources of contamination are removed, it can be seen that the degree of association of copper and iron with the solid, curdling, and soluble fractions of

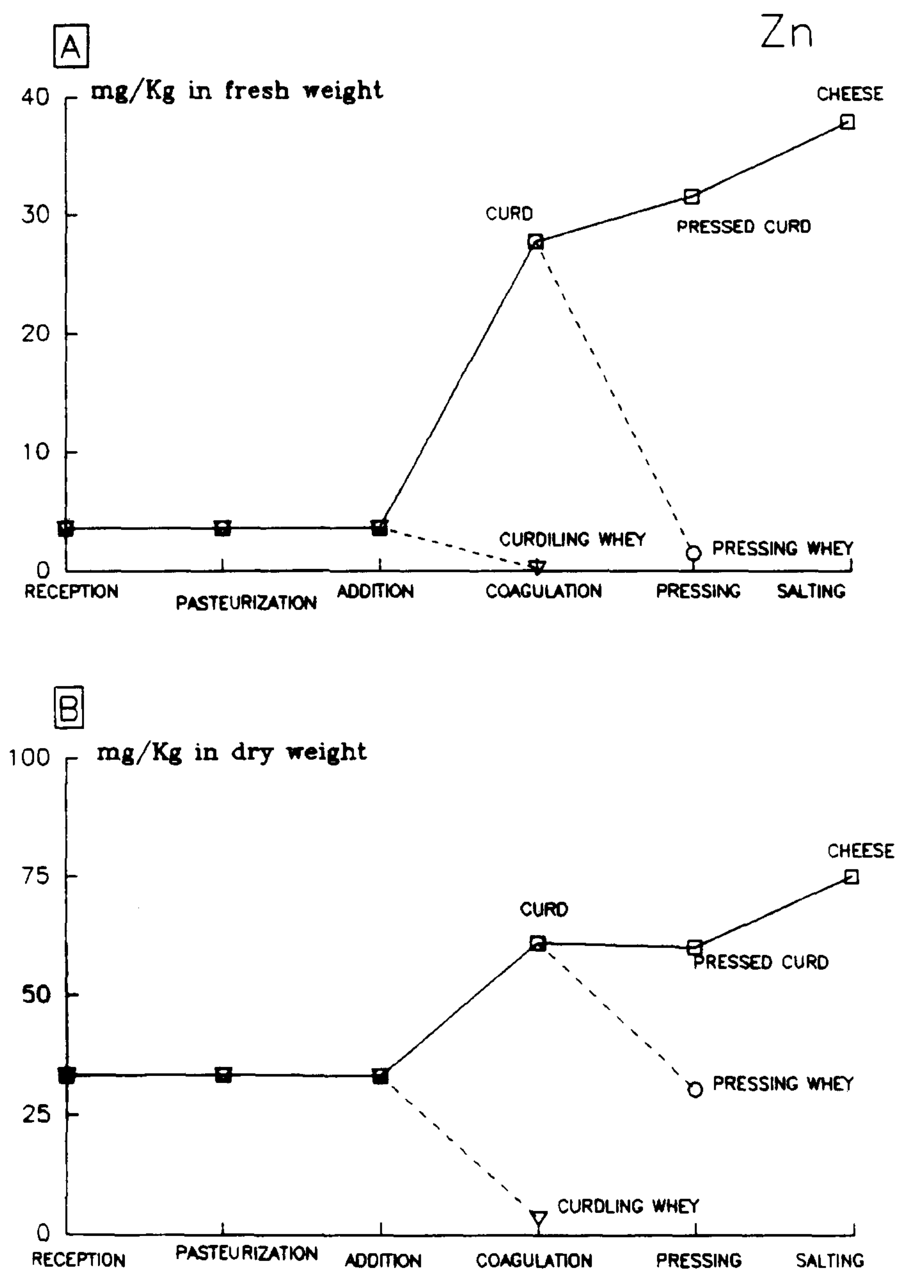

Fig. 3. Zinc evolution during the cheese-making process.

the milk are similar, since the curd and the curd whey show similar concentrations expressed as dry weight (Figures 1B and 2B). In the same way, a low association of these elements with the aqueous fraction of the milk can be assumed since their concentrations in the curdling whey expressed as fresh weight (Figures 1A and 2A) are much lower than those corresponding to the curd.

In the case of zinc, the degree of association with the curdling fraction of the milk seems to be higher, as can be seen from the strong dissociation between the curd and the whey, both as fresh weight (Figure 3A) and as dry weight (Figure 3B). These observations coincide with the association of these elements with specific fractions of milk made by Renner (1989) of 48%, 38%, and 85% for copper, iron, and zinc, respectively, for casein and fat (main fractions that form the curd) and with the degrees of filtration of these elements in milk indicated by Fischbach-Greene & Potter (1986). From these results, we deduce that the products used for curdling the milk in the cheese-making process do not modify the degree of association of the three elements investigated to any substantial degree. It would therefore be possible to establish theoretical calculations of the mean contents of these elements in cheese as a function of their mean contents in milks used for making this type of cheese. However, possible contamination during the process would have to be taken into account.

The estimate made in Spain of the consumption of this type of product is approximately 3·11 g per person per day in a total diet of 1387 g (Instituto Nacional de Estadistica, 1985). On considering the mean concentrations in fresh cheese for the three elements analysed, the daily intake estimate is 3·22 μg/day for copper, 20·9 μg/day for iron, and 117·8 μg/day for zinc. The density of nutrients presented by the three minerals studied in fresh cheese for a male adult, with an energy contribution of 1800 kcal/day (NRC, 1989) is from 24 to 36% for copper, 47% for iron, and 178% for zinc, which indicates that fresh Manchego-type cheese is a poor source of iron and copper, whereas it is an excellent source of zinc.

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