

# Determination of copper ions in jams produced in copper vessels

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## **Abstract**

Traditionally jams, jellies and marmalades have been made in copper vessels. Chefs and authors of culinary book sometimes argue that this metal makes “better” products because of its a high thermal conductivity. However copper consumption should be limited for safety reasons, some chemical forms being toxic. In view of finding best practices for the use of copper vessels, it is usefuf to know how much copper moves from copper vessels towards the jam, jellies and marmalades when they are being made. After the development of an analytical procedure of copper determination applicable to jams, jams were prepared in a copper vessel that was chosen to be dirty and oxidized (stored in a kitchen without being used for 5 years), in order to obtain jam samples contaminated with copper ions, and these jams were compared with jams made in the same copper pan after cleaning, and also with jams made in an enamel covered steel vessel; the analysis of a commercial jam advertised to have been made in a copper vessel was performed for comparison. For the interpre-

tation of the measurements of the copper concentrations, model jams were prepared from water, pectin and sugar, and the influence of pH was determined through the addition of acids. For all the samples, the determination of copper concentrations was performed by atomic absorption spectrometry after mineralization (using nitric acid) of jam samples. The dirty and oxidized copper basin did release as much as 48.41 (sd 17.80) mg of copper per kg of jam, whereas only 19.0 (sd 5.94) mg/kg of copper were found in jams prepared in the same vessel, after it had been cleaned using a traditional cleaning process (twice vinegar and salt, plus rinsing with water). However, in the cleaned vessel, the copper concentration rose to 38.3 (sd 6.01) mg/kg when the jam was stored in that same vessel for 4 hours. For comparison, the copper concentration in jams made in an enameled covered steel vessel was only 0.52 (sd 0.09) mg/kg. Analysis of the effect of pH showed

**Research Article**

that the copper concentration increases at low pH (1.3-3.0), but no chelating effect of citric acid was observed.

**Keywords**

jam, atomic absorption spectrometry, pH, pectins, copper

**Introduction**

Jams, jellies, marmalade and other similar products (gov.uk, 2003; 2020; DGCCRF, 2022) were initially introduced for preserving fruits after harvest, and such food products are still consumed because they are sweet, with nutritional and sensory appeal for human beings (Drenowski, 1995; This, 2008; Diligent, 2010). Such preparations were once obtained by thermal processing of fruits with honey (Rigaud and Saugrain, 1558), but nowadays they are more commonly made from table sugar (impure sucrose, generally obtained from sugar cane or sugar beets) (Tilly, 2010; Adetunji *et al.*, 2016).

During thermal processing, pectins are separated from plant cell walls, partially decomposed (This, 2009) and released, so that after cooling they make a gel (in which the aqueous solution and fruits pieces are trapped) if enough sucrose is present and if the pH is low enough (Allali, 2008; Adetunji *et al.*, 2016).

Pectins are polysaccharides that can interact with metallic ions from the surface of the vessels in which the jams are made, including copper ions  $\text{Cu}^{2+}$  from pans or basins which are traditionally used (Barbier and Thibault, 1982; Dauphin, 2018). Some cooks (This, 2009) explain that copper is a convenient material for the vessels used to make jams because of its high thermal conductivity ( $380 \text{ W.cm}^{-1}.\text{K}^{-1}$ , as compared to  $80 \text{ W.cm}^{-1}.\text{K}^{-1}$  for iron) (Lide, 2005), so that heat would be regularly distributed in the material, avoiding hot spots and

localized caramelization that would alter the flavour (Huawei *et al.*, 2013). A (non-edible) experiment that consists of adding  $\text{Cu}^{2+}$  ions to a jam (for example 10 g for 1 kg) shows that copper has the property to make the gel firmer:  $\text{Cu}^{2+}$  ions, as other divalent ions, can contribute to binding pectin molecules through electrostatic bonds between their carboxylate groups (Diligent, 2010).

However copper has biological effects. On one hand, it is needed for human life, being part of proteins and in particular enzymes (Frausto da Silva and Williams, 1991), and copper deficiencies can lead to anemia or osteoporosis. On the other hand, over-consumption of  $\text{Cu}^{2+}$  ions is responsible for kidney and liver damage through accumulation, and acute consumption of copper can trigger nausea, vomiting, abdominal pain and even death (Lindow and Elvehjem, 1929; Griffin, 1951; EFSA, 2018a; ANSES, 2019). Some copper salts (sulfate, carbonate and sub-acetate) inhibit endogenous enzymes (EFSA, 2015; Anant *et al.*, 2018).

The highest consumption of copper considered without risk, for a chronic exposure, is discussed in the toxicology community, but a 2022 consultation by the European Food Safety Authority is considering reducing the acceptable daily intake from 0.15 to 0.07 mg/kg of body weight (bw) (EFSA, 2022), whereas other sources recommend a value of 10 mg/day (Mahurpawar, 2015).

In order to investigate the question of how much copper can be consumed from jam, we restricted our study to using atomic absorption spectrometry (AAS) for the quantitative determination of the  $\text{Cu}^{2+}$  ions that moved toward the aqueous phase of jams in processing conditions that were analogous to the ones that are practiced in homes. Jams were made in dirty or clean copper basins, and the  $\text{Cu}^{2+}$  concentration were determined in these products. They were compared to  $\text{Cu}^{2+}$  concentrations in similar jams made in an enamel steel pan, in model jams (at different pHs), and also in a commercial apple jam made in a copper vessel.

## Materials and Methods

### Materials:

Model jams were prepared with white table sugar from Béghin-Say (purity > 99.5 %), “pectin NH Nappage” from Louis François Inc. (batch 18C3, 2021, later called “pectin”) and MilliQ water.

For experiments using apples (fruit of *Malus domestica*), always the same apple Boskoop variety was used (cultivated in France, growth season 2019). This choice of only one common variety was based on the fact that the high compositional variability of fruits would have prevented comparing different varieties, and also because this would have been a different goal than measuring the migration of copper. Three repetitions were done for each measurement.

All jams were made in the same copper vessel (De Buyer, 6200.38N, entirely made of copper). It was initially oxidized and dirty, as it had been stored for five years in the open air of a domestic kitchen, without cleaning (this was known because we could trace the last visit of the housekeeper who did the cleaning); the surface was no longer shiny, and it was somehow greasy. After jam samples were prepared directly in this vessel, without cleaning, a traditional method of cleaning was used, with spirit vinegar (8 %, DLP, Gretz) and white cooking salt (La Baleine Inc.).

The enameled steel vessel was a Beka pan 14396164, with enamel inside and outside, diameter 16 cm.

As a “commercial jam” from the company Albert Ménès (2021) was analyzed: it was made from 45 g of fruits (the variety of the apples used could not be known) for 100 g of jam, including sugar, apple juice, pectin, citric acid (quantities not given). On the packaging, it was advertised that the jam had been “cooked in a copper vessel”.

For pH measurement, a pH-meter (model 99621, Bioblock Scientific) with combined pH electrode (model 117 49798, Fisher Scientific)

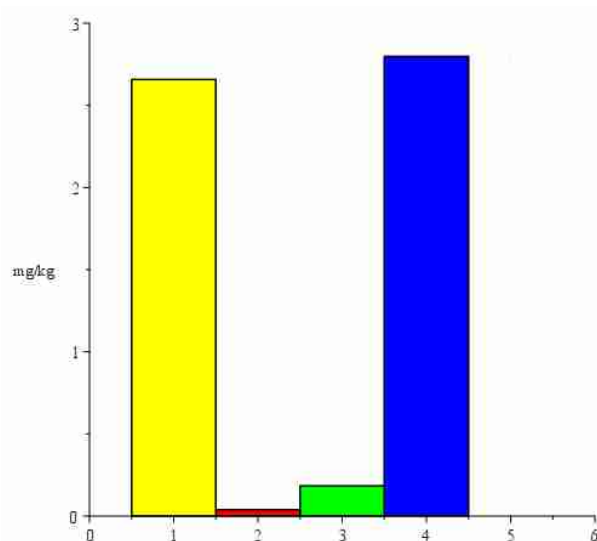


Figure 1. Quantities of copper (mg/kg) in the pectin used for the models (yellow); in the sugar used for all experiments (red); in a mixture of water, sugar and pectin (green); in the same mixture added with a known quantity of copper (blue). The error bars are present but they are too small to appear with this scale.

was used, after calibration with standard buffer solutions of pH equal to 4.01 and 6.87 (Schott buffers).

For sample preparation, hydrochloric acid was 37 % (AnalaR Normapur) from VWR. Copper pentahydrate sulfate was from Prolabo (analytical purity for analysis). Citric acid was 99.7 % pure on the basis of anhydrous product (Louis François Inc.). The water used for all experiments was ultrapure Milli-Q water (Merck-Millipore), for which the content in  $\text{Cu}^{2+}$  ions was checked to be under detection limits of the analytical method that was used. For mineralizations, nitric acid Plasma Pure, 67 – 69 % (SCP Science), and standard copper solutions 1000 mg/L (SCP Science) were used (Amard *et al.*, 1987).

For copper analysis, the spectrometer used was an Atomic Absorption Spectrometer model ContrAA 800D (Analytik Jena), type HR-CS-AAS. The analytical method is given below.

**Research Article**

**Methods:**

For all the experiments described here, all materials, products and reagents were weighed three times at each experimental step on a calibrated scale Mettler Toledo AG135, with a precision 0.0001 g.

For making the jams as well as the model jams, the copper vessel was held by two stands, tilted and heated by an IKA Ret. Basic Safety Control Heating system (precision 0.1 °C). Due to the low inertia of the small samples processed, the temperature had to be controlled manually during the thermal processing in order to avoid caramelization.

*1. Setting up the analytical method, and preparing model jams for interpretations.*

In order to develop the method used for Cu<sup>2+</sup> determination (sample preparation, mineralization, analysis), the content of Cu<sup>2+</sup> was studied in sugar, in the pectin product and in water, as well as in model jams (composed of all three ingredients) prepared in clean laboratory glassware. Here the goal was to analyze the quantity of Cu<sup>2+</sup> ions in these ingredients, without thermal processing. Some of these samples were used as negative references for the AAS determinations, but known quantities of Cu<sup>2+</sup> were also added to some of samples in order to follow their concentration during the analysis.

*2. Mineralization.*

AAS was performed after sample mineralization (Hoenig *et al.*, 1998; Bulska and Ruszcinska, 2017) with 5.0 mL of nitric acid 69 % added to each sample in a 50 mL mineralization tube. The tubes were heated in a digestion block DigiPrep Jr (SCP Science): the temperature was increased by steps of 10 °C until a temperature of 95 °C was reached (kept for 2 hours). Then MilliQ water was added until the volume was 50.0 mL, and cascade dilutions were performed until the concentration of the solutions could be determined using a

calibration curve (Danzer and Currie, 1998), based on the use of standard solutions with Cu<sup>2+</sup> concentrations equal to 0, 10 and 20 µg/L diluted with nitric acid (5 %).

*3. Atomic absorption analysis (AAS)*

AAS using the ContrAA 800D (Analytik Jena) spectrometer, was applied to the mineralized samples, looking for Cu<sup>2+</sup> ions in the various samples (List *et al.*, 1971; Welz, 2004). The oven was used as an atomizing source (electrothermal atomization): this had the advantage of detection limits lower than for traditional spectrometers (0.02 µg/L for Cu<sup>2+</sup>) (Hoenig and De Kersabiec, 1995; Vandegans, 1997).

For each sample, 20 µL of each sample were injected in the graphite tube (cuvette) with pyrolytic coating, and the heating was performed after a thermal program in three main steps: drying, decomposition and atomization (Dawson *et al.*, 1968). During the process, drying is performed in 3 sub-steps, the last one being at the temperature of 110 °C; the vaporization of the solvent has to be completed in order to avoid losses by projections, because of the fast increase of the temperature at the beginning of the next step (Bradfield and Spincer, 1965). The decomposition process (pyrolysis) at 900 °C achieves mineralization, simplifies the sample matrix, avoids smoke, molecular vapors and the presence of other constituents formed during organic mineralization. Atomization at 2000 °C dissociates the residual matrix, generating an atomic fog that includes the copper atoms.

The element of interest (here copper) is then selected by the high resolution monochromator at the specific wavelength used for analysis. Its concentration relates to the light attenuation after atomic absorption (Beer-Lambert Law). The line of copper at 324.754 nm was used for Cu detection, according to the standard protocol of the spectrometer (Analytik Jena, 2021). The

**Research Article**

analyses were performed under argon flux (Hoenig and De Kersabiec, 1995). Pure solutions of known concentration in  $\text{Cu}^{2+}$  ions were used to validate the method. All samples were analyzed 3 times. Finally the results given by the spectrometer were analyzed using the software *Maple 2021* (Maplesoft/Waterloo Maple Inc), in particular for the calculation of uncertainties and the statistical treatments.

**4. Making jams in the uncleaned copper vessel.**

The first group of jams produced were made (using a process that was comparable to culinary ones) in the copper vessel which was purposely kept uncleaned (dirty, oxidized and greasy), because we wanted to determine a maximum  $\text{Cu}^{2+}$  concentration in jams. Seven samples were produced in different places of the vessel, because we looked for an estimation of the variability of  $\text{Cu}^{2+}$  release as a function of the position on the vessel. Each apple was cut in pieces of about 1 g each, in parts of the fruits as comparable as possible for each experiment, in order to minimize compositional variability of the fruit samples. In order to get about 1 g of jam available for

analysis after cooking, taking into account the water evaporation and the losses on the vessel, we prepared about 2 g of each sample, from about 1200 mg of sugar and 800 mg of fruit (for all products used, the quantities used were precisely known, and they differed from the target value by less than 10 mg); the pH was measured and found equal to 3.11. The duration of the thermal treatment was 10 min for all samples, with the same heating power (Wilson *et al.*, 1993).

**5. Cleaning the vessels.**

After this first experiment, the copper vessel was cleaned twice, *i.e.* rubbed using white cooking salt (La Baleine Inc.) and spirit vinegar (8 %, DLP, Gretz), as advised by a traditional culinary protocol still followed today by chefs and pastry chefs (Bonnechère, 1904). The final rinsing was performed with milliQ water, because copper contamination of water was to be avoided. Before using the enameled steel pan, it was washed with hot water and soap, rinsed with tap water, boiled twice with MilliQ water and rinsed with cold MilliQ water.

*Table 1. The copper content in the various materials analysed (average values and standard deviations in mg/kg).*

<u>Models (figure 1)</u>	<u>Pectin</u>	<u>Sugar</u>	<u>Water + sugar + pectin</u>	<u>Water+sugar+pectin + copper</u>
	2.657 (sd 0.0001)	0.0386 (sd 0.003)	0.184 (sd 0.003)	2.798 (sd 0.002)
<u>Jams (figure 2)</u>	<u>Oxidized vessel</u>	<u>Cleaned vessel</u>	<u>Storage in cleaned vessel</u>	<u>Enamel</u>
	48.41 (sd 17.80)	19.0 (sd 5.94)	38.30 (sd 6.01)	0.52 (sd 0.09)
<u>Effect of pH (figure 3)</u>	<u>HCl, pH 1.5</u>	<u>HCl, pH 2</u>	<u>HCl, pH 3</u>	<u>Citric acid, pH 2</u>
	176.90 (sd 12.7)	104.80 (sd 12.7)	119.70 (sd 2.2)	120.13 (sd 12.74)

**Research Article**

**6. Making jams in the same vessel after cleaning.**

Then various samples were prepared in the following way:

- (1) samples of the same batch of fruit which were prepared in the same vessel after it was cleaned by the traditional method,
- (2) model samples (made of water, sugar, pectins) were prepared in the cleaned copper vessel but with a storage for 4 hours after thermal treatment (this time was chosen because it is much longer than the processing time, and it is what could happen in kitchens),
- (3) samples of an apple jam that was bought at the grocer's and for which the only information given was that it had been prepared "in a copper pan".
- (4) some jams were also prepared from the same batch of fruit but in the enameled steel vessel, for comparison.

**7. Exploring the effect of pH.**

Finally, the influence of the pH on the release of  $\text{Cu}^{2+}$  in jams was also explored, adding to model jams various concentrations of hydrochloric acid or citric acid (citric acid is not only an acid, but also a chelating agent, sometimes also used for extracting pectins) (Dufrénoy, 1954; Glusker, 1980; Di Palma and Mecozzi, 2007; Kermani *et al.*, 2015; Martinez *et al.*, 2018). For these experiments, the acid was added while the pH was recorded, until it was changed from 3.11 (corresponding to the previously measured pH of "natural" jams made in this study) to 1.50, 2.00 and 3.00 in groups of three replicates.

**Results**

For preparing the interpretation of the results of jam analysis, the  $\text{Cu}^{2+}$  concentration in water, sugar and pectin was determined, as well as in model jams made of these three components. Also, the  $\text{Cu}^{2+}$  concentration was measured in

apples (Diligent, 2010). These results are shown in Figure 1.

It is in the pectin preparation that was used that the higher  $\text{Cu}^{2+}$  concentration was found (2.798 mg/kg, sd 0.002), compared to 0.0386 mg/kg (sd 0.003) for sugar. The copper observed in pectin alone can be found proportionally in model 1 (water, sugar, pectin): the difference between the measured value and the calculated one (on the basis of the proportions of the ingredients) is not significant ( $p < 0.05$ ). Moreover the study of the model added with copper (as pentahydrate copper sulfate) showed that mineralization was producing solutions in which all the added copper could be determined. In other words, the addition of known quantities of copper to the water-sugar-pectin mixture (recovering test), with successive dilutions, validated the method with external standardization (the  $R^2$  factor for the calibration lines was 0.9993).

The quantities of  $\text{Cu}^{2+}$  in jams made after using various vessels are graphically displayed on Figure 2. For jams made respectively in the uncleaned vessel, and in the same vessel after cleaning, the average  $\text{Cu}^{2+}$  concentrations were 48.41 (sd 17.80) and 19.0 (sd 5.94) mg/kg respectively. The  $\text{Cu}^{2+}$  concentrations in samples of jams made in the uncleaned vessel are significantly higher than the  $\text{Cu}^{2+}$  concentration in jams that were stored for 4 hours in the cleaned vessel (38.30 mg/kg, sd 6.01). For the jams that were made in enameled steel, the  $\text{Cu}^{2+}$  concentration was much lower (0.52 mg/kg, sd 0.09).

Finally we were interested in measuring the  $\text{Cu}^{2+}$  concentration in a commercial jam, for which the packaging said that the jam had been prepared in a copper vessel ("Préparée manuellement dans un chaudron en cuivre ouvert, cette gelée est élaborée avec du pur jus de pomme", *i.e.* "hand made in an open copper vessel, this jelly is produced from pure apple juice"): the  $\text{Cu}^{2+}$  content was found to be 0.59 mg Cu/kg (sd 0.002). A one-way ANOVA test with  $F$  ratio 19.6 ( $p$ -value 0.00003) shows that there are significant differences between all samples produced in dirty and oxidized vessel, samples

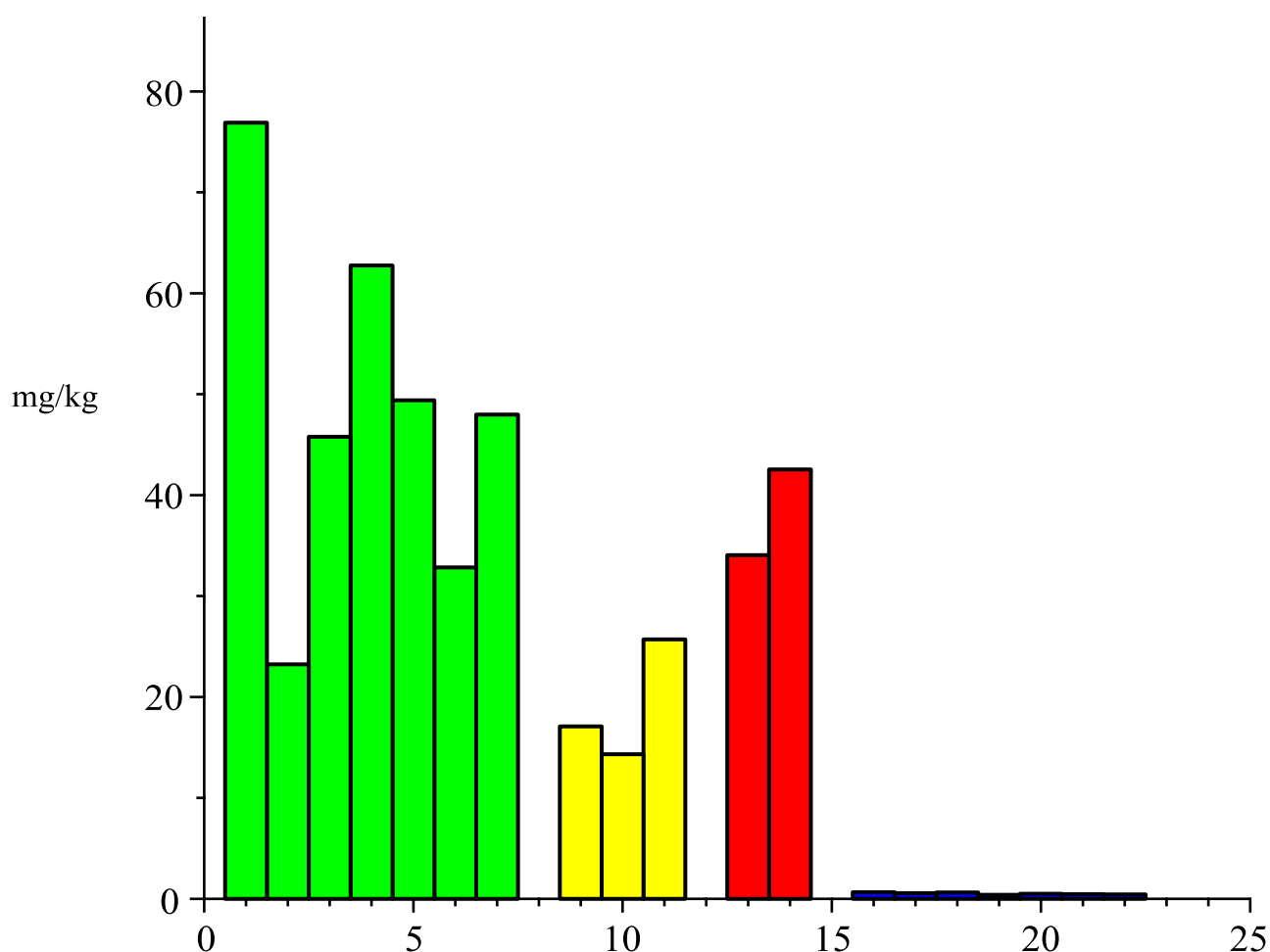


Figure 2. Copper concentration (mg/kg) in jams made in a copper vessel that was not cleaned before use (green), in jams made from the same copper vessel after home cleaning (yellow), in jams stored for 4 hours at room temperature in the cleaned copper vessel (red), in jams that were made in enameled steel (blue). Various measurements are shown, in order to give a better appreciation of the variability in copper concentration; the error bars about the copper concentration for each sample are too small to appear in this view.

made in cleaned vessel, samples made in a cleaned vessel with 4 hours storage, and samples made in enameled steel.

Finally, in order to estimate the influence of the pH of the jam on the  $\text{Cu}^{2+}$  concentration, hydrochloric acid and citric acid were added to samples before thermal treatment (Kondratiev *et al.*, 2021). Citric acid was chosen because the citrate ion is a chelate of divalent ions,

such as  $\text{Cu}^{2+}$ . The sample at pH = 1.5, with the addition of hydrochloric acid, displayed a high  $\text{Cu}^{2+}$  concentration (176.90 mg/kg, sd 12.7). At pH = 3, again adjusted with hydrochloric acid, the  $\text{Cu}^{2+}$  concentration was reduced to 119.70 (sd 2.2) mg/kg. The  $\text{Cu}^{2+}$  concentrations at pH = 2 are similar for the samples added with hydrochloric acid and with citric acid (Figure 3).

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**Discussion**

The determination of  $\text{Cu}^{2+}$  by AAS is a standard method (Amiard *et al.*, 2003). In our study, preliminary experiments were needed only to (1) determine the required dilutions for mineralized samples, (2) validate the analytical method in the particular conditions for jam analysis, (3) produce calibration curves to be used for the determination of copper in the various samples. The obtained calibration lines were always going through the origin of concentrations, with  $R$ -squared higher than 0.999. Because the standard deviation of the values being measured included the origin of absorbances, the rule that indicates that the calibration curve should be forced to pass through the origin was applied (Danzer and Currie, 1998). The limit of detection was thus equal to 0.08 mg/kg.

First the analytical method was applied to the exploration of the pectin and sugar components of the models. The technical sheet of the "pectin" used (Louis François, 2021) did not give the quantity of copper in the product; it only indicated limits for lead (" $< 5$  ppm"), mercury (" $< 1$  ppm"), arsenic (" $< 3$  ppm") and cadmium (" $< 1$  ppm"). The values that we determined for  $\text{Cu}^{2+}$  ions in the pectin product used (2.657 mg/kg, sd 0.001) are of the same order of magnitude as for these regulated contaminants. They can also be compared to the maximum authorized limit (4 mg/kg) for the release of materials in contact with food (List, 1970; DGCCRF, 2017). For the sugar, the  $\text{Cu}^{2+}$  concentration was found equal to 0.0386 (sd 0.0008) mg/kg, but this is less than the detection limit of the method.

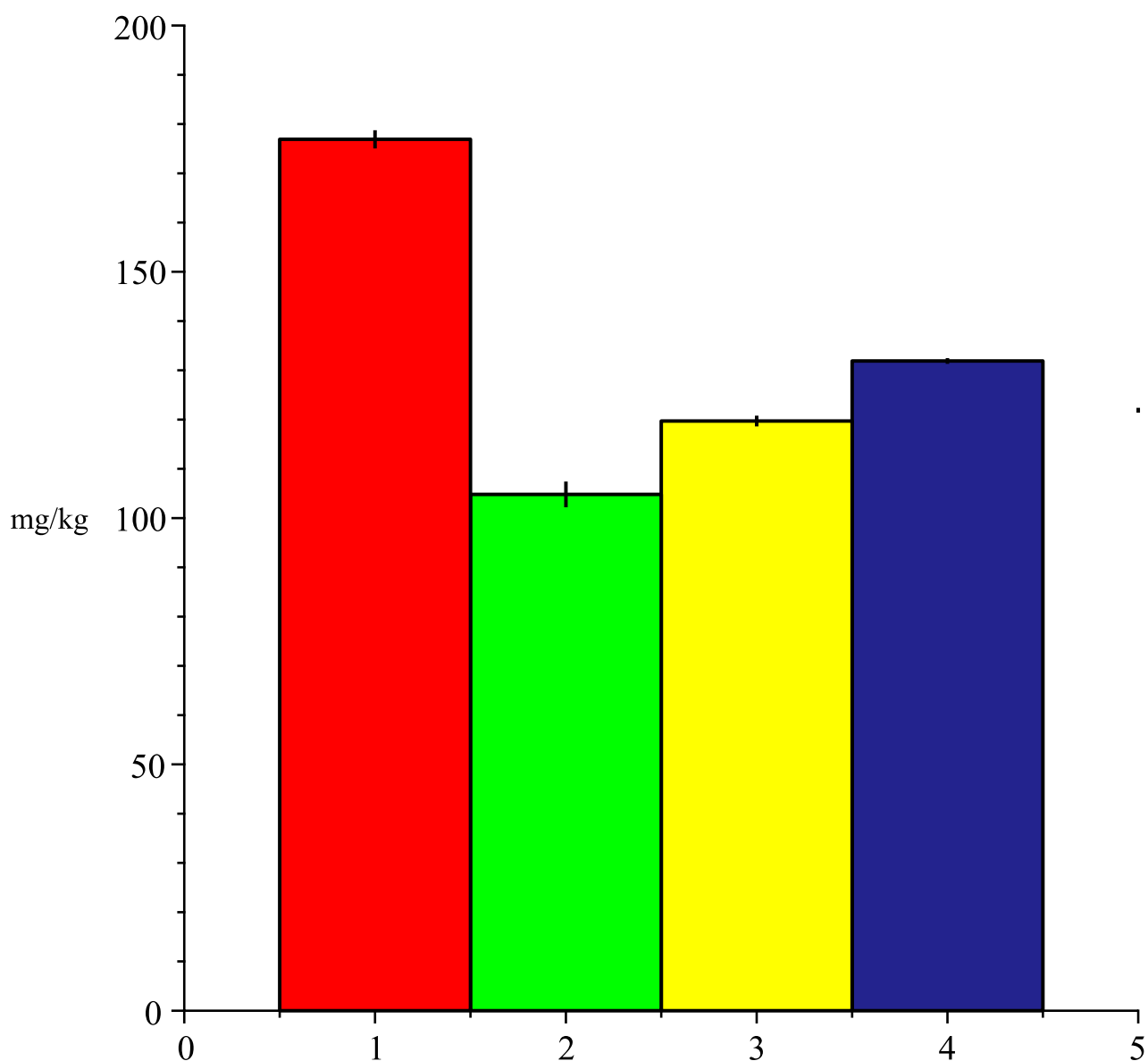
In order to interpret the relatively high concentration of  $\text{Cu}^{2+}$  in "pectin", it is useful to remember that pectins (8-11 g of pectins/kg fresh plant tissue) are primarily located in the middle lamella of the primary wall of plant cells, where their concentration reaches 40 % (Massiot *et al.*, 1989). This would correspond to a maximum quantity of 0.02 g of pectins in the jam samples.

In order to determine how much  $\text{Cu}^{2+}$  such an amount of pectins could "trap", one can observe that the pectin molecules are considered to be an

alternation of "hairy" and "smooth" zones, made of 30-35 % of D-galacturonic acid residues (3,4,5,6- tetrahydroxy - tetrahydro - pyran - 2- carboxylic acid, D-AGal) (Massiot *et al.*, 1989); the average molar mass is about 100,000 (from some to many hundreds of DAGal residues) linked with  $\alpha$ -(1 $\rightarrow$ 4),  ${}^4\text{C}^1$  conformation (Aspinall, 1980; Voragen *et al.*, 1995). The bonds between D-AGal residues are axial-axial (Braccini *et al.*, 1999), so that the homogalacturonanes molecules have a right helix conformation, with 2-3 monomers per turn (Braccini *et al.*, 1999; Pérez *et al.*, 2000). A certain proportion of carboxylic groups is esterified with methanol: the esterification degree can reach 60 % of the carboxylic acids groups for the pulp of apples or the skin of lemons. Also, the pectin molecules contain rhamnogalacturonic chains: every 80-100 residues (Massiot *et al.*, 1989), the homogalacturonic chain includes L-rhamnopyranose units (6-methyl-tetrahydropyran-2,3,4,5-tetraol, Rha). These chains are thought to be made of repeats of the dimeric motive  $[\rightarrow 4)\text{-}\alpha\text{-D-AGal-(1}\rightarrow 2)\text{-}\alpha\text{-L-Rha-(1}\rightarrow ]$  (Renard *et al.*, 1995). Depending on the particular origin of pectins, the proportion of substituted rhamnose residues can change; it is about 60 % for sugar beets (*Beta vulgaris*), but only 10-50 % for carrots (*Daucus carota* L.) (Voragen *et al.*, 1995). Finally conformational studies (Braccini *et al.*, 1999; Pérez *et al.*, 2000) showed the possible positioning of calcium ions  $\text{Ca}^{2+}$  on the main chain, so that we can use such data for determining the maximal capacity of pectins to bind not only calcium, but also copper, through structures having the shape of square based pyramids (Braccini *et al.*, 1999; Pérez *et al.*, 2000).

In this assumption of carboxylic acid groups of pectins linked to  $\text{Cu}^{2+}$  ions, the maximum copper content in pectins can be calculated: from a known mass of pectin, assuming a certain molar mass, one can determine the number of D-AGal residues in a molecule (dividing the molar mass of pectin by the one of D-AGal), hence the total number of D-AGal residues in the mass of pectin. Assuming 4 carboxylic acid groups per





*Figure 3. Copper concentration (mg/kg) in jam samples with (1) hydrochloric acid at pH=1.5 (2) hydrochloric acid at pH=2, (3) hydrochloric acid at pH=3, (4) citric acid at pH = 2.*

copper bridge, as for calcium bridges, and a proportion of copper bridges equal to the proportion of calcium bridges (0.33, according to Bracchini *et al.*, 1999 ; Jeantet *et al.*, 2006), one can determine that the maximum quantity of bound  $\text{Cu}^{2+}$  ions would be 12 g/kg of pectin, which is much higher than what we measured. Hence, for sure the  $\text{Cu}^{2+}$  concentration was higher in

pectins than in sugar, but this quantity is much lower than the maximum possible content.

About the determination of  $\text{Cu}^{2+}$  in jams, it can be observed that in the past (Alexandre-Bidon, 2005) copper vessels were introduced in kitchens because they did not break as earthen vessels would, and they did not rust, unlike iron tools. However the toxicity of “verdigris” led the

**Research Article**

culinary world to line the copper tools with tin, except for vessels used in jam making, sugar cooking, and green vegetable boiling (Kühn, 1970 ; Schell *et al.*, 2012).

For example, Albert (1838) writes (personal translation): “When making jams, one should mix using a wooden spoon, making a figure of 8. One should not use an iron spatula, because they would scrape the bottom of the basin and take off small particles of copper. Copper basins can be used safely if they are very clean and cooled jam should not be stored in them, because the acids from fruits, which don’t attack copper when hot, can dissolve when cold.”

Audot (1847) adds (personal translation): “Copper vessels that are not lined with tin should be used (earthen vessels can lead to burning or give bad flavours). People that do not have a special basin can use a cauldron, but it has to be cleaned very carefully. One has to heat very energetically. Nothing should be stored in the basin, because of the formation of verdigris. This is why it is important to pour the jams into pots as soon as they are cooked”.

Babinsky, an engineer who wrote a 1284 page cookbook (1907), mentions a flavour that would be due to copper: “For jams made of red fruits, it is best to use enameled basins that don’t give fruits any acridness, as it is often observed for non-tin lined copper basins.”

However, to our best knowledge, no author of any cookbook gave any reference to scientific works that would justify what they wrote about copper toxicity and about the practices that they recommend to avoid, and it is a result of both empiricism and public hygiene programs that cooks slowly restricted copper tools to cooking green vegetables, caramel and jams. For green vegetables, copper basins are called greening basins, and they release  $\text{Cu}^{2+}$  ions that replace magnesium ions at the center of the tetrapyrrolic ring of chlorophylls, generating copper chlorophyllins responsible for a bright green color (Valverde and This, 2008; Burrows, 2009). For caramel, tin cannot be used, because the lining would be damaged by high temperatures, hence the use of pure copper. For jams, as said in the

introduction, the educational experiment that consists of adding about 5 grams of copper sulfate to the jam made from two oranges and the same mass of sugars (This, 1997) showed that the gel being made is considerably reinforced by binding copper ions to carboxylate groups (it is solid, instead of flowing).

About the experiments of making jam in various parts of the oxidized copper vessel, one can first observe a very high variability of the  $\text{Cu}^{2+}$  concentrations depending on the position in the vessel where the jams were prepared: the difference between the maximum and the minimum  $\text{Cu}^{2+}$  content is 1.1 times the average copper content. As said, the vessel was stored vertically, hung by a handle, before being used, and this could lead to changes of the surface aspect and composition, the fat droplets and dust could deposit more on one side (the lower one).

Knowing the average quantity of  $\text{Cu}^{2+}$  that moved into the solution, it is interesting to compare it to the quantity of metal that could be oxidized, at the surface of the basin. If we assume an area  $A$  of the surface in contact with the jam, with an oxidized thickness  $e$  (volume  $V = A \cdot e$ ) that is assumed to be hydrated hydrogenocarbonate  $\text{Cu}_2(\text{OH})_2\text{CO}_3$  (FitzGerald *et al.*, 2006), with a density of  $6 \text{ g/cm}^3$  (Lide, 2005; American Elements, 2021), then the  $\text{Cu}^{2+}$  determination in jam leads to calculating a corrosion thickness of 0.04 mm, to be compared to the published value of  $\sim 0.01$  mm per year. Indeed, the basin that we used for our experiments was not cleaned for 5 years: it was stored in the free atmosphere, in a kitchen, its surface was not shiny and there was some dust on it; of course, because it was hung by one handle before being used in this study, part of it (the upper half, when it was hung) was cleaner than the lower one, on which grease droplets could deposit, changing the exchange with air.

The comparison of the  $\text{Cu}^{2+}$  concentration of jams made in the oxidized vessel and in the same vessel after cleaning indicates that indeed the significant difference is due to oxidation during storage. At this step, it could not be

**Research Article**

decided if the large variability (sd 5.94, for an average value of 19.0) of the  $\text{Cu}^{2+}$  content in the cleaned vessel was due to the variability of the plant material or to different oxidation states of the vessel, but the other experiments in enameled steel showed that the second assumption is more likely.

Unfortunately the small quantities of plant material that we could use in order to make all the experiments (with the same fruit) did not allow for the determination of  $\text{Cu}^{2+}$  in various parts of the apple (this can be the topic of a further study, even if it is already measured, see Nour *et al.*, 2010), but the analysis of jams made in the enameled pan gave indications: the  $\text{Cu}^{2+}$  concentration was 0.52 mg/kg, with 0.7000 g of plant tissue, 0.8000 g of sugar, 0.5000 g of water. This leads to a  $\text{Cu}^{2+}$  content of 0.144 mg/kg for the apple and the copper that migrated from the enamel. This quantity is much lower than the one that was measured in jams cooked in copper. For this first series of measurements, the possible variability of plant tissue has a lower influence than the one due to the copper basin: the variation coefficient is about 37 %, but always for very large values, almost 10 times higher than the release limit of materials in contact with food. This phenomenon can be explained by the heterogeneous aspect of the basin, during the first series of experiments (before cleaning).

About cooking in enameled materials, the low  $\text{Cu}^{2+}$  concentration is likely due to the particular process for making this material (Demont *et al.*, 2012; ISO, 2018). Ceramics are inorganic and non-metallic materials often made from mixtures of clay, earthen elements, powders and water; once the ceramic has been shaped, it is fired at high temperature in a kiln, and decorative, waterproof, paint-like substances known as glazes can be applied subsequently. Vitreous and porcelain enamels are used as coating on steel/cast-iron articles in order to prevent corrosion of the metal underneath and to provide a closed, abrasion-resistant and physiologically safe surface that is fit to come into contact with foodstuffs (Benzesik *et al.*, 2018). Ceramics are regulated at EU level by the directives

84/500/EEC and 2005/31/EC. The only minerals regulated under these directives are lead and cadmium, with limits imposed for the release of these two elements into food and rules for migration testing using a 4 % acetic acid solution as a simulant. Nothing is said about copper from enamels in the documents of the European Enamel Authority. For DGCCRF (2017), the technical data do not concern enameled materials, but only when there is an inorganic lining or when this lining is hybrid, organo-mineral (such as given by sol-gels processes). For enameled material that contain no lead or cadmium, there is no legal obligation to check the extraction of these elements, and, accordingly, we did not get information from the maker (CETIM, 2019). A report from the European Commission is giving a limit of released copper of 4 mg/kg (Simoneau *et al.*, 2016). This value can be compared to our determination in the jam made in enameled pan, of 0.52 mg/kg (average value).

For this value, the variations (CV 0.17) should be less the effect of enamel than from the plant material, for which the copper content is of the same order of magnitude as from enamel (Aprifel, 2022; Bednarek *et al.*, 2007 ; Lidow *et al.*, 1929).

About  $\text{Cu}^{2+}$  that moved towards jam when stored in the copper basin, we could see that storing the jam in contact with copper is often said to be dangerous, with more  $\text{Cu}^{2+}$  released, and this is what we observed, in spite of the fact that the basin had been cleaned. For interpreting this result, it is interesting to quote a preliminary experiment in which raspberry (*Rubus idaeus*) juice was stored in a cleaned copper basin: after two hours of storage, the color of the metal (visual observation) was much brighter below the level of liquid (pH 2.4) than above. This means that either the cleaning step performed in kitchens is not efficient, in terms of  $\text{Cu}^{2+}$  ions suppression, or that  $\text{Cu}^{2+}$  ions are formed by the attack on the copper metal by the acidic liquid.

The pH decrease has two effects: first protons can interact with the metal of the vessel, changing the quantity that is released in the jam,

**Research Article**

and secondly it improves the firmness of the gel. Hence the fruits having a high concentration in pectins and a low pH are better candidates for making firm jams. However this increases the copper concentration in jams.

The control of the pH of the jam preparation was interesting because pH determines the protonation of carboxylate groups, so that hydrophobic parts of pectins can associate by hydrophobic forces (when the sugar concentration is between 40 and 65 %) (Belitz *et al.*, 2009). Copper, the noblest of the 3d transition metals, offers corrosion resistance in near-neutral and alkaline environments owing to the passive film spontaneously formed on its surface (Langley *et al.*, 2018; Speck and Cherevko, 2020). However, the passive film undergoes local degradation in acidic environments, especially in solution that contains ions such as  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  (Izquierdo *et al.*, 2017; Touzé and Cougnon, 2018). The research on copper corrosion has been a subject of concern in material sciences for decades in order to better predict it and to prevent its deterioration. It is known that the acidity of the jam promotes the attack of metal (Rigg and Marflitt, 1971; Moreira *et al.*, 1993; Isquierdo *et al.*, 2017). For dilute solutions of hydrochloric acid, the time rate of copper transformation into copper ions is a function of acidity, copper ion concentration and dioxygen availability, the main phenomena being the attack of copper metal by cupric ions, and the reoxidation of the resultant cuprous ions by dioxygen. In jam making, the ingredients for these two reactions were present.

In home made jams, the natural acidity of fruits, which contain mainly citric, galacturonic, isocitric, malic, oxalic, quinic, succinic, tartaric acids among others (Walker and Famiani, 2018) is sometimes increased by adding lemon juice. Citric acid ( $pK_a$  values 3.1, 4.8 and 6.4), with chelating properties, could block the action of free  $\text{Cu}^{2+}$  ions, reducing the attack (Glusker, 1980; Di Palma and Mecozzi, 2007; Martinez *et al.*, 2018). This effect seems to be based on an electrostatic effect, between copper ions and citric acid: when the global negative charge of the citric acid increases, an increase of the electrostatic

interaction with positive  $\text{Cu}^{2+}$  ions is produced. However the results given here show that the average  $\text{Cu}^{2+}$  concentration in samples at pH 2, because of the addition of citric acid, is not significantly different ( $p = 0.09$ ) from the samples at pH 2 with the non-chelating hydrochloric acid. Before assuming a competition between the chelating effect of citric acid and the formation of copper bridges, one will have to consider the kinetic conditions, important for the attack of copper (Martinez *et al.*, 2018).

Finally the low concentration of  $\text{Cu}^{2+}$  in the jam from the industry is surprising, in particular when compared to the jam that we made in enameled steel. One possibility (to be explored further) is that the frequent use of the same vessel, as it is likely to be the case for a jam producing company, can avoid the formation of a corrosion film that would prevent the release of  $\text{Cu}^{2+}$  ions. However a solution to avoid the presence of such ions (except when coming from pectin and sugar) while keeping a possibility of bridging pectins in order to make firm jams, is to use stainless steel vessel and add divalent calcium ions ( $\text{Ca}^{2+}$ ) in the jam.

Finally the acceptable daily intake of  $\text{Cu}^{2+}$  is now proposed to be 0.07 mg/kg of body weight (bw) (EFSA, 2018b; EFSA, 2022). Using the data given here, one can calculate that the corresponding quantity of jam made in a "dirty" vessel, for a 70 kg person, would be only 70 g per day. However, in order to get a better appreciation of the exposure, the work shown here should be continued with more studies, in many different directions: the influence of acidity on the jams, the influence of the various metallic divalent ions on the firmness of the jam, the determination of the time course dissolution of copper in the jams, the influence of the processing time on copper dissolution, the influence of the particular nature of pectins on copper dissolution.

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**Research Article**

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