DEVELOPMENT OF A METHOD FOR EVALUATING FLOOR DRY-CLEANABILITY FROM WHEAT FLOUR IN THE FOOD INDUSTRY

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Abstract: Many productive processes are characterized by inadequate protocols of sanitation that increase the possibility of proliferation of microbial contaminants, especially on surfaces. The use of this method for evaluating the degree of floor cleanability in agri-food companies is important not only to reduce the risk of contamination of products, but also to provide companies with a tool to identify critical issues. The method is based on the usage of bicinchoninic acid assay (BCA) in a solution at a 1:50 ratio of Cu^{2+}/BCA, which is ideal for detecting the amount of proteins contained in wheat flour residues on industrial flooring. Spectrophotometric analysis allowed identifying maximum absorbance values at 562 nm for different protein concentrations, while the construction of a regression function led to the definition of the intervals of evaluation corresponding to different degrees of cleanliness from residues of wheat flour. The results of the absorbance curves, obtained by applying the proposed evaluation method to six tiles commonly used in agri-food buildings, showed the clear persistence of food material on two tiles with surface relief. In particular, such tiles showed a higher presence of proteins, with a level of contamination 440% higher. Furthermore, a robotic system was designed to standardize the cleaning method commonly employed in agri-food companies to remove solid particles from flooring.

Keywords: agri-food building, cleaning, contamination, floor, food safety.

Introduction

Inadequate cleaning of agri-food facilities causes the proliferation of microorganisms, which may be pathogenic. The physico-chemical characteristics of food favour the colonization and growth of bacteria and fungi that may even cause life-threatening diseases in human beings (Dzieciol and others 2016). Many productive processes are characterized by inadequate protocols of sanitation that increase the possibility of proliferation of microbial contaminants, especially on surfaces. This results in a considerable impact on public health: every year, in industrialized countries, up to 30% of the population suffer from foodborne diseases (WHO 2007; Porto and others 2015; Bridier and others 2015).

Recent studies have confirmed the direct relation between the amount of nutrients found on surfaces after cleaning and initial microbial growth (Blel and others 2010; Kumar and Anand 1998). Low amounts of such substances reduce biofilm formation and make microorganisms vulnerable to disinfectants (Garrett and others 2008). As a result, lower quantities of sanitizing substances are required.

It is now common knowledge that four main factors affect cleaning: temperature, chemical action, time and mechanical action (Burkinshaw and others 2011). The last mentioned, which corresponds to the energy spent to remove the residues of food from surfaces, measures the cleanability of a surface and mostly depends on the physico-chemical interactions between the contaminant, the contaminated surface and the cleaning material (Hoek and Angarwal 2006; Jullien and others 2003; Katainen and others 2006).

Floors are the surfaces most at risk of contamination, often because of inadequate cleaning protocols that are characterized by low levels of sanitation favouring biofilm formation (Faille and Carpentier 2009). Large amounts of food are often accidentally spilled on floors during processing phases and if not adequately removed may generate a substantial accumulation of substances mainly composed of proteins, starches (Otto and others 2016) and lipids, which are the principal sources of nourishment for the metabolic activities of microorganisms (Donlan and Costerton 2002).

In the last few years, the industry of building components has been promoting research on new easy-to-clean materials and surfaces for floors that may meet the market, requirements, and at the same time support environmental sustainability in cleaning and sanitizing food facilities thanks to the limited use of detergents. Standardized tests for the evaluation of floor cleanability, *e.g.*, stain resistance and sanitation tests (ISO 10545-14:2015), as well as experimental non standardized methods are regularly applied to the most commonly used materials. Schöler and others (2009) included an optical analysis of the starches found on the surface during the action of a solution of NaOH under pressure; however all of these procedures require the use of detergents and/or disinfectants.

Nevertheless, such methods do not allow evaluating the cleanability of surfaces in the specific production sector as they are mainly calibrated for civil buildings. For example, in the case of the stain resistance test, existing regulations provide for a simple visual evaluation of the presence of staining agents, while the agri-food industry requires an evaluation of the presence of traces, even not visible, that may favour the formation of bacterial colonies.

The objective of this project was to define a standard method for the evaluation of the dry-cleanability of floors from food residues, in particular, from wheat flours used in the food industry. The method was based on the measurement of protein residues by means of a solution of copper sulphate pentahydrate and bicinchoninic acid. A specific application to six stoneware tiles with different surface finishing, commonly used in agri-food facilities, was developed to validate the method.

Materials and methods

The method included four procedural steps:

- Contamination of a reference surface with a known quantity of flour;
- Cleaning of the surface through a standardized procedure;
- Sampling of the residue of contaminant;
- Quantitative evaluation of the residue of contaminant

The material to be evaluated was contaminated by evenly distributing, by means of a powder sprayer, 1 g of type 00 common wheat flour, which is commonly used in the food industry and whose composition is shown in Table 1, on a 20 cm × 20 cm sample of the flooring surface.

Table 1 - Composition of the flour contaminating the surface to evaluate

Substances	Quantity (g/100g wheat flour)
Fats	1.10
Carbohydrates	75.00
Sugars	0.52
Fibres	2.50
Proteins	8.31
Sodium chloride	0.01
Other	12.56

The wheat flour was distributed on the analysed surface in a controlled environment at a temperature of around 25°C, with humidity of no more than 80% and air velocity not exceeding 1 m s^{-1}. The whole quantity of flour was evenly distributed on the surface.
The second step of the procedure included the cleaning of the surface by scrubbing with a cleaning cloth, a procedure which is usually carried out manually in agri-food facilities before any cleansing and sanitization of surfaces.

1.1. Proposal of an apparatus to simulate the dry cleaning of the surfaces and sampling of residual wheat flour

In order to simulate correctly the actions performed during manual cleaning, and, above all, to standardize the method and make it replicable, a special mechanical apparatus was designed.
It was composed of a rectangular 100 cm-long and 50 cm-wide aluminium frame on which guides were fixed to drive a 13 cm × 42 cm sliding block supporting the cleaning cloth. The sliding block was driven by an electric engine fed by a programmable electronic card that allowed changing its speed and direction as well as the overall number of cleaning cycles (0 1).
The pressure exerted by the sliding block was controlled by a digital sensor placed on the interface between the cloth and the analysed surface in order to obtain a constant value of 269.51 N m^{-2}, which corresponded to the average of the pressure measurements taken on the floor sweepers during the usual sweeping of over 20 different operators. The motion of the sliding block was programmed to reverse its direction only once during the cleaning cycle, so as to simulate the operator's movement during the manual cleaning procedures.

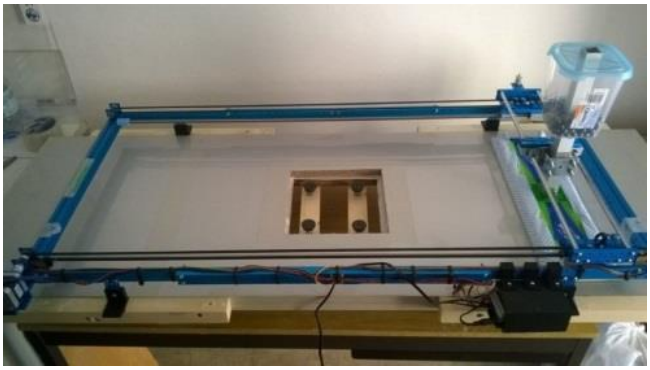

Figure 1 - Automated mechanical system for the simulation of the tile cleaning

The cloth used to simulate cleaning operations, which was fixed between the sliding block and the surface to analyse, was obtained from the Kimberly-Clark© Professional WYPALL X80 Hydroknit. It is a cloth commonly used in the cleaning of food companies (Figure 2).
An important feature of such a mechanical prototype is its transportability, which allows using it not only on samples of tiles in the laboratory but also on site, directly on floors. Furthermore, its programmability favours its adaptation to various production sectors and different methods of cleaning. In order to perform laboratory tests also on limited portions of surfaces, such as single tiles, a specific support was designed to ensure the correct laying of the sample. It was made of a non-porous material and had a size which did not affect the regular movement of the sliding block and allowed firmly fixing the system. The sample of the surface to analyse was lodged in a hole at the centre of the slide bed. Moreover, the surface was made planar to the support by a manual levelling system. After cleaning the surface with the automated system, the sample was washed thoroughly with a jet of 100 ml distilled water, which was completely recovered, together with the suspended residues of flour, to perform subsequent analyses.

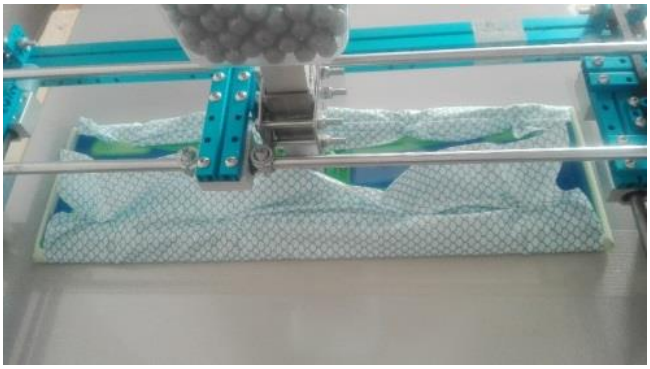

Figure 2 - The sliding block

1.2. Evaluation of residual traces of proteins.

The method of bicinchoninic acid (BCA) (Brady and Macnaughtan 2015) was used to detect residual flour after cleaning. Such a method allows detecting the presence of proteins. It is commonly used during the monitoring phases of HACCP (Hazard Analysis Critical Control Point); it is easy to apply and provides a colorimetric evaluation in about 10'. It is based on the chemical reaction between copper sulphate and the proteins found in the solution that reduce Cu^{2+} to Cu^{+} in proportion to the quantity of proteins. Cu^{+} is then chelated by the bicinchoninic acid, which originates the colour change of the solution ranging from green to purple-crimson.
In order to establish the optimal ratio between the copper sulphate pentahydrate used in a 4% aqueous solution and the bicinchoninic acid, which is useful to find even small traces of proteins on the surfaces, four test solutions, having Cu^{2+}/BCA ratios equal to 1:25, 1:50, 1:100 and 1:200, respectively, were compared (Biradar and others 2016).
Four 4 ml samples were taken from each of the four solutions and then 180 mg, 840 mg, 2530 mg, 9020 mg of flour were introduced, which approximately corresponded to the following quantities of proteins: 15 μg, 70 μg, 210 μg and 750 μg, respectively.
After 10', 3 ml of test solution were taken from each test tube and then introduced in cuvettes for spectrophotometric analysis (Figure 30). Analyses were performed by means of a UV-1600PC spectrophotometer, which allowed obtaining the absorbance curves.

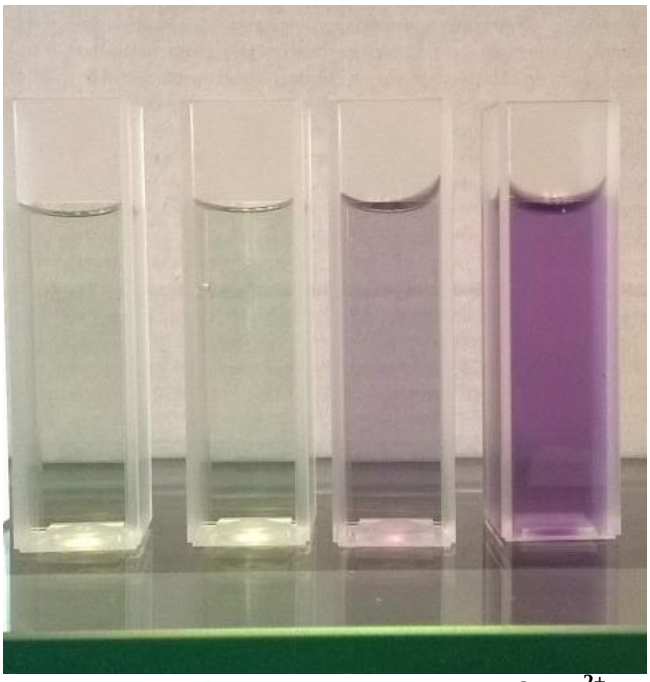

Figure 3 - Test solution at a 1:50 ratio of Cu^{2+}:BCA

The following figures show the spectrum curves and the values of maximum absorbance of the four test ratios for the four quantities of proteins (Figure 4-7) (Table 2)

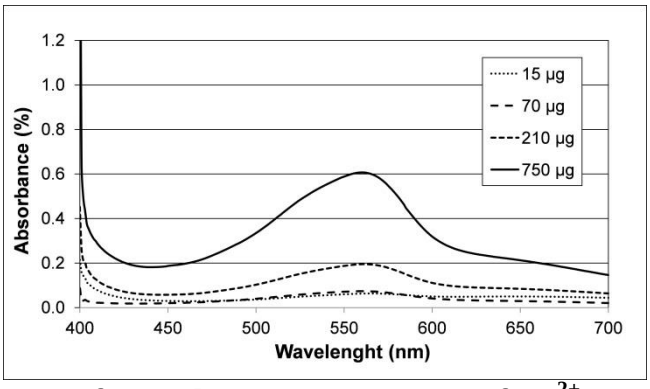

Figure 4 - Spectrum curve for a solution at a 1:25 ratio of Cu^{2+}/BCA containing the four different quantities of proteins

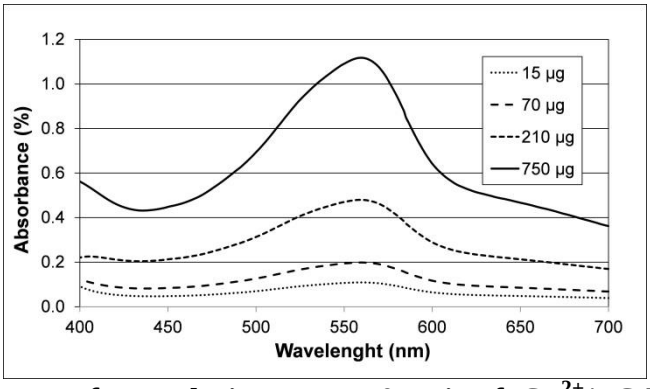

Figure 5 - Spectrum curve for a solution at a 1:50 ratio of Cu^{2+}/BCA containing the four different quantities of proteins

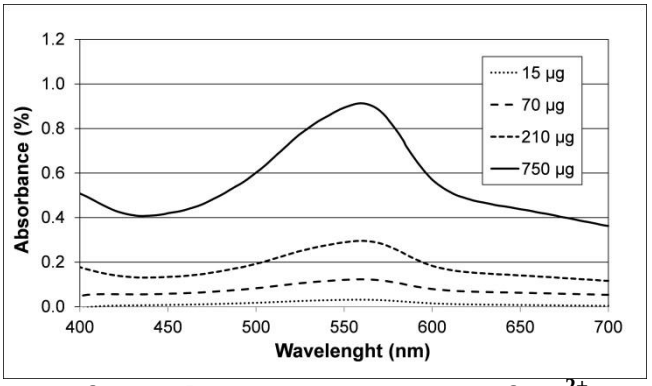

Figure 6 - Spectrum curve for a solution at a 1:100 ratio of Cu^{2+}/BCA containing the four different quantities of proteins

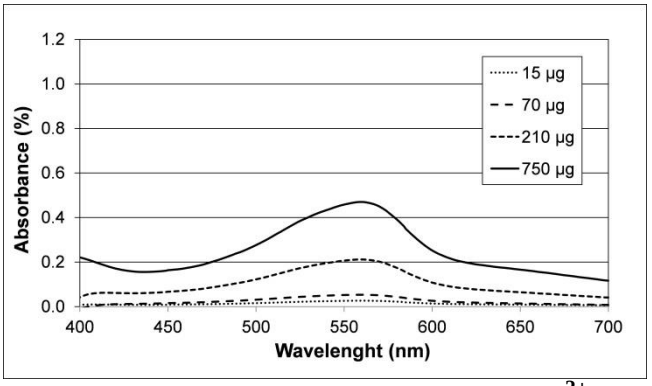

Figure 7 - Spectrum curve for a solution at a 1:200 ratio of Cu^{2+}/BCA containing the four different quantities of proteins

Table 2 - Maximum absorbance values at 562 nm related to the four Cu^{2+}/BCA solutions containing four different quantities of proteins

	Proteins (µg)			
Cu^{2+}/BCA Ratio	**15**	**70**	**210**	**750**
1:25	0.06355	0.07393	0.19509	0.60643
1:50	0,10898	0,19813	0,47877	1,11529
1:100	0.03136	0.12280	0.29531	0.91142
1:200	0.02664	0.05322	0.21120	0.46892

The comparison of the solutions having different Cu^{2+}/BCA ratios led to the conclusion that the 1:50 ratio was the solution that allowed better discriminating the different quantities of proteins. The regression function [1] was constructed for this solution to relate the quantity of proteins to the absorbance value at a maximum wavelength of 562 nm.

$$r(x) = a - b \cdot exp^{-c \cdot (x)^m} \quad [1]$$

Where r (x) was the content of proteins corresponding to the maximum absorbance value x at 562 nm measured through the spectrophotometric analysis, m was the form factor equal to 1.3352, c was the scale factor equal to 0.0003245, a was equal to 1.2367 and b was equal to 1.1414 (Figure 8).

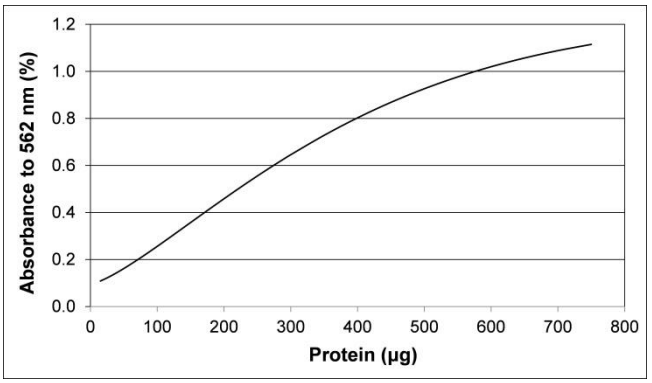

Figure 8 - Regression function of absorbance values related to proteins in a solution at a 1:50 ratio of Cu^{2+}/BCA

2. Results and discussions

With a view of validating the method proposed, it was applied to a series of six 20 cm × 20 cm stoneware tiles (Table 3), commonly used in the food industry (Figure 9).

Table 3 - Visual characteristics of the tile surface ~~finish~~

Type	Colour	Texture
A	Grey	Smooth
B	Light brown	Smooth
C	Grey	Smooth
D	Light brown	Smooth
E	Light brown	With cross-shaped elements in relief
F	Light brown	With lozenge-shaped elements in relief

Tiles featured oversized thickness to ensure high shock-resistance, since they are commonly used for heavy-traffic floor areas. They had a single compact body mass of noble clays sintered at 1250 °C to obtain a material with a high resistance to thermal shocks and chemical attacks. Their surface was slip-resistant and tiles E and F, in particular, were characterized by a finish with elements in relief that improved the grip between the floor and the sole.

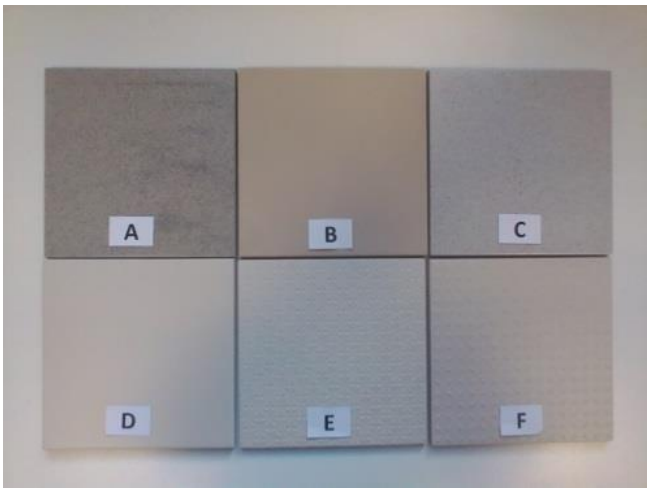

Figure 9 - Stoneware tiles analysed

2.1. Surface roughness of the analysed tiles

Specific measurements of the roughness characteristics of the surface were taken for the six tiles. Standard roughness parameters R_a, R_q and R_{max} (Sedlaček and others 2011; Flint and others 2000), which are commonly used to describe the characteristics of the surfaces, were not enough to define the contact properties of the material, because similar textures could be characterized by different values of roughness parameters. As a result, parameters R_q (root mean square roughness), R_p (maximum height of peaks), R_v (maximum depth of valleys), R_t (maximum peak to valley height), and R_z (ten point height mean) (Table 4) were also measured.

Table 4 - Tile roughness values

	Tile					
Parameters	**A**	**B**	**C**	**D**	**E**	**F**
Ra (μm)	1.891	2.655	1.840	2.125	6.748	6.743
Rp (μm)	5.118	6.663	5.563	5.750	17.478	17.291
Rq (μm)	2.339	3.301	2.293	2.626	8.298	8.278
Rt (μm)	13.869	21.574	13.758	14.442	46.214	46.462
Rv (μm)	5.142	8.937	4.587	5.402	16.072	15.717
Rz (μm)	10.261	15.599	10.150	11.151	33.550	33.008

2.2. Application of the BCA method

The most difficult step of the method proposed was the sampling of the residue of wheat flour after the standardized cleaning process with the mechanical device. The method adopted was the complete washing of the tiles by means of a 100 ml jet of distilled water, which allowed completely recovering the residual flour from the surface without using any detergent that could alter the final evaluation.

Then, 100 ml of test solutions with Cu^{2+}:BCA ratio equal to 1:50 were added into each washing water recovery container. After about 10', 6 ml of test solution were taken from each washing water container and introduced into a centrifuge to accelerate the sedimentation of the flour particles in the solution, which, if still suspended, could alter the measurement of the absorbance spectrum. Centrifugation was carried out at 172 x g for 2' at a temperature of 15 °C.

Finally, a 3 ml sample was taken from each test solution for the spectrophotometric analysis, which allowed constructing the spectrum curves and the concentration of the analysed solutions (Figure 10) (Table 5).

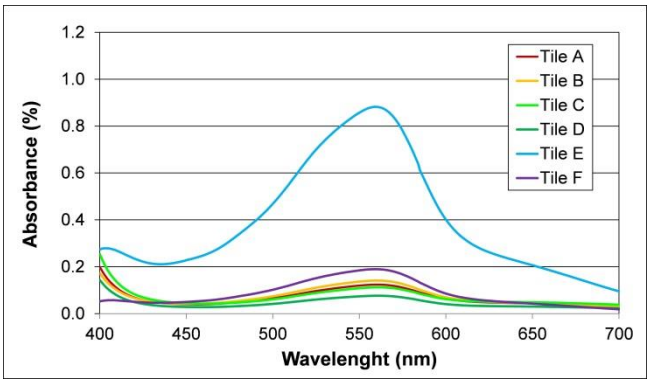

Figure 10 - UV-V is the absorbance curve of the samples of washing water taken after the dry-cleaning of tiles

Table 5 - Maximum absorbance values at 562 nm of the samples of washing water taken after the dry-cleaning of tiles

	Tile					
	A	**B**	**C**	**D**	**E**	**F**
Absorbance [%]	0.1617	0.0862	0.1207	0.0789	0.9583	0.2179
Concentration [ppm]	0.4118	0.2173	0.2993	0.1958	2.3259	0.5289

Following the use of the cleaning apparatus, a visual inspection of the analysed tiles highlighted the clear persistence of food material on the slip-resistant texture and, in particular, on tile E, due to the surface relief which affected the contact with the cleaning surface and hindered the removal of the residual flour. This observation was confirmed by the results of the absorbance curves obtained by applying the proposed evaluation method to the six tiles. Such tiles showed a higher presence of proteins on tile E than on tile F, with a level of contamination 440% higher.

Furthermore, tiles E and F showed a high roughness value (R_a > 6 μm), about 3.2 times higher, on average, than that of the tiles without elements in relief.

Among the tiles having a texture with no element in relief, D was characterized by the lowest value of contamination. It showed an absorbance equal to about 0.0789 % and a protein concentration equal to 0.1958 ppm. It was followed by C, B and A (Figure 11).

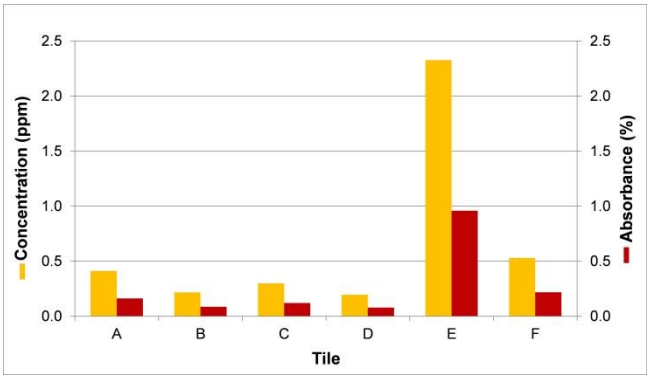

Figure 11 - Maximum absorbance at 562 nm and protein concentration ~~by stoneware tile~~ of the samples of washing water taken after the dry-cleaning of tiles

3. Conclusions

In order to show the degree of cleanliness of a surface in a clearer and more immediate manner, the results of the spectrophotometric analysis can be expressed through a qualitative evaluation scale. Regression function [1], which describes the correlation between the maximum absorbance value of the solution measured at 562 nm and the protein concentration, allows defining the intervals of evaluation corresponding to different degrees of cleanliness from residues of protein (Table 6).

Table 6 - Evaluation scales of the degree of flooring cleanliness in relation to absorbance values at 562 nm

Degree	Absorbance (%)
Clean	≤ 0.129
Doubtful	0.130 – 0.217
More than doubtful	0.218 – 0.295
Dirty	0.296 – 0.646
More than dirty	0.647 – 0.925
Very dirty	> 0.925

Such a scale can be easily and quickly applied and could be used during monitoring and control steps or to compare different surfaces.

The application of the method proposed in this paper allowed validating its effectiveness and relatively simple use. The importance to have a standardized method of analysis that could be easily applied and allow evaluating the cleanability of surfaces from wheat flour residues and, in particular, of floors in agri-food facilities, plays a crucial role in the search for design solutions for sustainable buildings. For instance, tests showed that the elements in relief on the surfaces of floor areas were an obstacle to cleaning, even if they could improve grip. Actually, the wide range of products and components offered by the market of building materials requires a careful evaluation and analysis of specific uses. A floor could be suitable for a cheese factory but not for a mill, since both the nature of the contaminant and the level of hygiene and safety to guarantee in relation to the specific product are different. The physico-chemical characteristics of the contaminant play a fundamental role in cleaning procedures and, above all, in how its persistence on the surfaces is examined.

In this paper, the BCA method was used. It seems to be suitable to detect traces of contaminants containing proteins but it is not equally suitable to highlight the presence of contaminants that do not contain such molecules. Therefore, it is important to continue to search for and establish the most appropriate methods and techniques to detect not only the presence but also low quantities of different types of molecule. Finally, the application of the method highlighted the complex interactions existing between materials, their surface finish and contaminants. Further studies and research can better evaluate the links and correlations between materials and contaminants to direct technical and design choices towards more suitable and appropriate materials in relation to the specific food production process.

Acknowledgements

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