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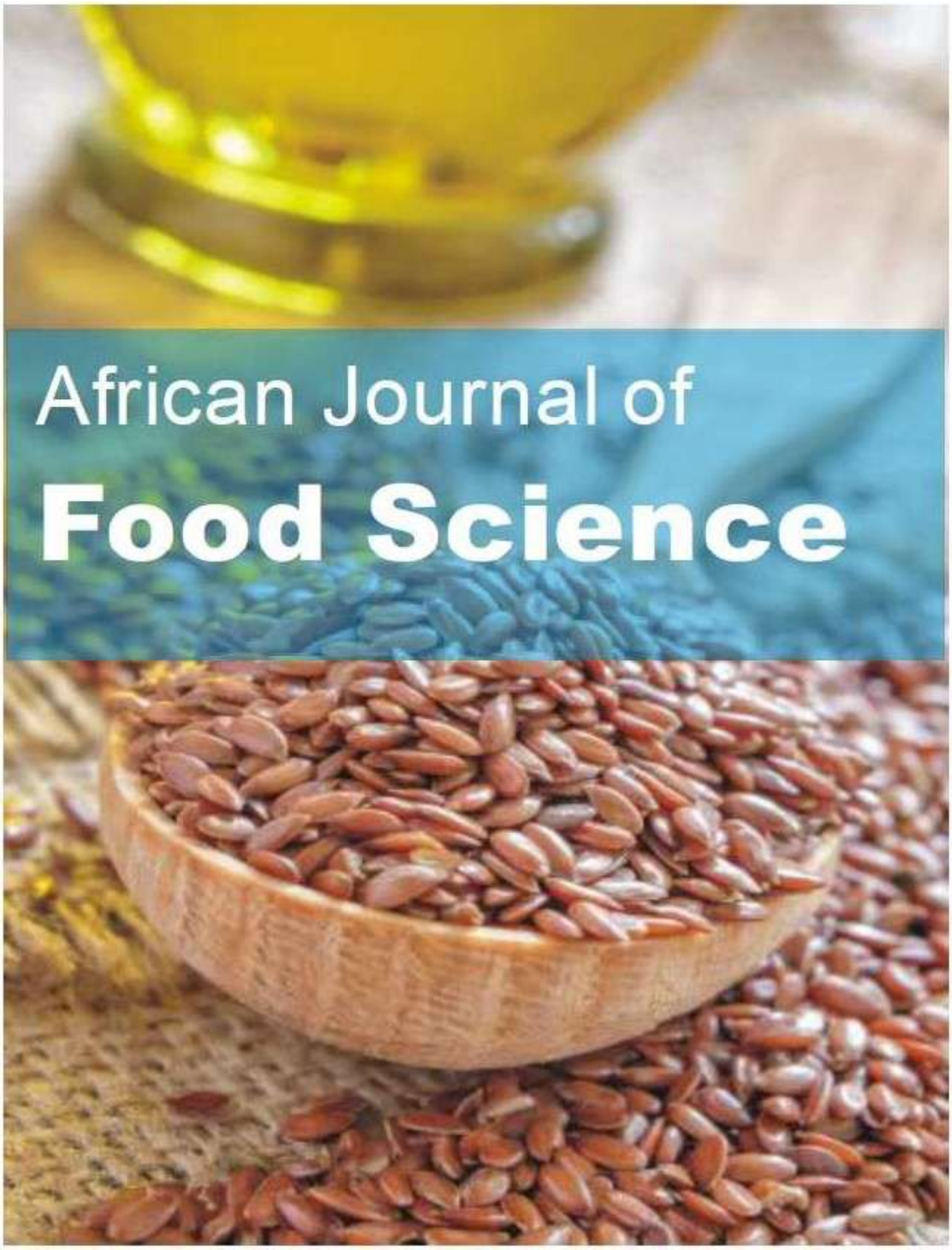

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African Journal of Food Science

Full Length Research Paper

Effect of smoking technologies on nutritional values and concentration levels of polycyclic aromatic hydrocarbons in smoked fish (*Mormyrus caschive* and *Oreochromis niloticus)* of Terekeka, South Sudan

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Effect of smoking technologies on nutritional quality and concentration levels of polycyclic aromatic hydrocarbons (PAHs) in *Mormyrus caschive* and *Oreochromis niloticus* were determined to enable adoption and utilization of suitable facility that maintains good quality for sustainable supply of nutritious fish products in South Sudan. A total of 72 fresh *M. caschive* and *O. niloticus* were purchased, of which 24 were iced for reference and the other 48 were divided into two groups for pit and *chorkor* smoking. Experimental smoking of fish samples was conducted twice in a randomized design. Fish samples were analyzed for nutritional values using standard methods of the association of official analytical chemists and levels of PAHs using gas chromatography-mass spectrometry. Results revealed; the two smoking methods concentrated crude protein, fat and ash contents. However, fish smoked using *chorkor* had significantly higher nutritional values than fish smoked using pit kiln. About seven types of PAHs comprising of low and medium molecular weight were recorded from the two smoked fish species. Naphthalene and fluorene were the two dominant PAHs with fish smoked using pit kiln having significantly higher concentration levels of naphthalene (5.86±4.16 µg/kg) and fluorene (3.83±0.10 µg/kg) than fish smoked using *chorkor* oven. The study concluded that *chorkor* oven has better quality smoked fish than pit. Hence, its utilization and adoption for artisanal fisheries in South Sudan is recommended.

Key words: Nutritional values, food quality and safety, smoking techniques, polycyclic aromatic hydrocarbons (PAHs).

INTRODUCTION

Fish post-harvest handling provides livelihoods and income to many people in the world, especially countries

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gifted with aquatic resources (FAO, 2016). Fish processing and preservation are important because fish is perishable and prone to quality decline immediately after harvest and to deter economic, quality and nutrient losses (Msusa et al., 2017). Proper handling of fresh fish is vital to achieve the best quality and highest profits (Muhame et al., 2020). Regardless of these immense contributions, the fishing industry suffers from considerable post-harvest losses which are valued at 30% in dry season and 40% in rainy season (FAO, 2015). Fish post-harvest losses have intense adverse effect on rural fishing population whose income and wellbeing often depend on fisheries activities (Adeyeye and Oyewole, 2016). Lack of appropriate infrastructure for fish post-harvest handling is a major challenge facing rural fishing communities of South Sudan. As such better preservation facilities and techniques are required to reduce the effects of fish post-harvest losses (Famurewa et al., 2017).

Amongst the customary preservation techniques of fermenting, salting and sun drying, fish smoking is the leading type of fish preservation method used in Terekeka County. This is due to the fact that most consumers prefer smoked fish to fermented, sun dried and salted fish. Besides, lack of suitable processing and preservation infrastructures have made fisher-folks to extensively use smoking to preserve their catches which could not be delivered to far markets in fresh form. Hygienic and efficient fish smoking indeed, preserve the nutritional quality and safety of smoked fish due to antioxidants and preservatives; phenol and formaldehyde contained in the smoke (Magawata and Musa*,* 2015*;* Pemberton-Pigott et al., 2016). However, fish smoking may lead to deposition and accumulation of carcinogenic PAHs in the products as a result of fuel source and the method used in processing fish.

Concisely, PAHs are a class of organic compounds that are typically formed and released during incomplete pyrolysis of organic matter such as waste or food, during food preservation, industrial processes and other human activities (Abdel-Shafy and Mansour, 2016; Lee et al., 2019). They are a large group of organic compounds with two or more fused aromatic rings (Erawaty-Silalahi et al., 2021). Although PAHs have a relatively low solubility in water, they are extremely lipophilic. Besides, most of the PAHs with low vapor pressure in the air are readily adsorbed on some particles (Erawaty-Silalahi et al., 2021). There are more than 100 PAHs, of which 16 of them are categorized as PAHs of health concern (Yusuf et al., 2015; Tran-Lam et al., 2018), and are used to determine the presence and levels of carcinogens in food. However, the European Food Safety Authority (EFSA) recommended that the concentrations of benzo[a]pyrene (BaP), and the sum of concentrations of four PAHs: benzo[a]pyrene (BaP), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), and chrysene (Chry) (PAH4), be considered as a reference for determination of PAHs in food (Puljic´ et al., 2019). According to the regulations on maximum limits for PAHs contamination in food (EFSA, 2008), which complied with the European Commission (EU) regulation no.835/2011 (EC, 2011) as amended, the maximum permissible limits for concentrations of BaP in meat and fish products is set at 2 µg/kg and the sum of PAH4 concentrations set not to exceed 12 µg/kg (Wet weight). As such, the use of PAH4 was adapted in 2014 to be the most suitable indicators of occurrence of PAHs in food (Zelinkova and Wenzl, 2015; Puljic´ et al., 2019). Due to different techniques of food preservation, a lot of studies have been done to determine the levels of PAHs in food using PAH4 as indictors of carcinogenic potency. This is done to ensure safety of preserved food and protection of consumers' health. However, none has been done for preserved fish products of South Sudan.

It is noted that, due to variation in smoking parameters, PAHs levels in smoked fish may exceed the maximum acceptable limit of 2 µg/kg for benzo[a]pyrene, 12 µg/kg for the sum of PAH4 recommended by the European Commission (EC, 2011). During smoking, the concentration levels of PAHs may increase. Besides, the levels of PAHs contaminants are associated with detrimental human health effects (cancer causing effects). Additionally, poor heat control during processing may lead to increased temperature that degrade nutrients hence, reducing nutritional values of smoked fish products. The present study therefore, assessed the effect of smoking technologies on nutritional quality and concentration levels of PAHs in smoked *Mormyrus caschive* (Family: Mormyridae, Common name: Elephant snout) and *Oreochromis niloticus* (Family: Cichlidae, Common name: Nile tilapia) with the aim of adopting and utilizing suitable smoking facility that maintains the nutritional values and safety of smoked fish for sustainable supply of fish products in South Sudan.

MATERIALS AND METHODS

Study area

The study was done in June, 2018 in Terekeka County of Central Equatoria State, South Sudan. The area is located approximately, 52 miles north of the capital city, Juba on the western part of River Nile (Benansio, 2013). Its geographical position is within latitudes of 5˚23'N and longitudes of 31˚48'E (FAO and WFP, 2019). The county occupies an estimated area of 10,538.232 km^2 with population projection of 246,483 (South Sudan Centre for Census, Statistics and Evaluation, 2018). Terekeka has tropical climate with relatively small periodic variations in humidity, temperature and wind the whole year (Climate-data.org., 2018). It receives rainfall from the months of March to May and August to November. Annually, an average rainfall of 907 mm is received in the area. Terekeka is having two dry periods; between the months of December to February and June to July with average annual temperature of 27.7°C. It is in the dry season where most fisher-folks are actively participating in fishing and other support activities. However, abundant periods of fish harvest occur in the months of June, July and August mostly after flood inundation recedes. It is

Figure 1. The two smoking ovens used during the study.

within this period that majority of fish processors mainly women and children are actively involved in fish processing and preservation particularly, smoking of their catches.

Study design

Experimental smoking of the two species; *M. caschive* (Family: Mormyridae; common name: Elephant snout) *and O. niloticus* (Family: Cichlidae; common name: Nile Tilapia) using pit and *chorkor* facilities was done twice using a complete randomized design. The *chorkor* facility used for the experiment measured 2 m long, 1 m wide and 1m high. It has three chambers with trays attached in each compartment. This smoking oven is made of unburnt bricks, interior plastered with clay soil and perforated hard iron sheets as roof. The base has two inlets for aeration and smoke production. Its smoking chamber has flexible door that remains shut except during checking periods. A customary pit erected beside *chorkor* oven performance. The pit kiln measured; 1 m long, 0.5 m wide and 0.5 m high as used by fisher-folks in Terekeka. Logs are usually put at the edges for wire mesh to sit. In the process of smoking the fish, flat iron sheet was placed on top of the mesh to cover the samples (Figure 1).

Sampling, processing and analytical procedures

In total, seventy two (72) fresh *M. caschive* and *O. niloticus* were bought at Sur-num fish landing site located about one kilometer East of Terekeka Town. Fresh fish samples were preserved in ice-cold containers immediately after harvesting at the fishing grounds. Fresh samples procured for experimental smoking were processed following traditional procedures. A purposeful smoking of fish samples using *chorkor* and pit ovens was done at Terekeka fish landing station. Fish smoking took an average time of 8 h in *chorkor* and 20 h in pit kiln. From the total fresh fish, 12 samples of each species were stored at 4°C in ice-cold containers before being transported by bus to the laboratory of which, 6 specimens from each species were destined for proximate chemical composition and PAHs analyses, respectively. The other 48 samples were divided into two groups for pit and *chorkor* experimental smoking using *Acacia seyal,* the main tree species used by fisher-folks for smoking fish in the area. All fishes were carefully washed to remove slime, descaled, eviscerated and rewashed thoroughly with clean water to remove blood. Cleaned samples of fish were immersed in freshly prepared salt solution (a mixture of 100 g salt in 10 L of clean water) for 15 min followed by draining for 15 min. Fire was set in pit and *chorkor* ovens to produce smoke heat. The cured fish samples were randomly loaded on the trays and wire mesh in a *chorkor* and pit ovens, respectively. The preferred temperature of 60 - 80°C was maintained manually by the help of a thermometer until all the fish samples were smoke dried. During smoking, the position of fish samples in the trays were changed in *chorkor* to attain uniformity and turned upside down in pit kiln for uniformity of the smoking process. Thereafter, the smoke dried samples were cooled for 12 h at ambient temperature of 20°C. Smoke dried fish were enclosed in an aluminum foil, labeled, packed in carton boxes, and transported by bus to the laboratory in Makerere University, Uganda for analyses.

Sample preparation for analyses

From each sample, one hundred grams (100 g) of smoked fish muscle was removed from the edible parts, grinded to powder form using blender, and the weighed samples were labeled and stored in deep freezers at -18°C for proximate chemical analyses. One hundred grams of fresh fish muscles from each sample were taken as control and prepared following the same procedures as the treatment samples.

Chemical composition analyses

After homogenization of the weighed edible portion of each specimen, the following determinations were performed in triplicate by following the standard methods for the Association of Official Analytical Chemists (AOAC, 2005); moisture content determined by the weight reduction method, total crude protein determined by digestion, distillation and titration using micro-Kjeldahl method, crude fat determined by solvent extraction procedures in a Soxhlet system an ash content determined by incineration procedures in a muffle furnace using weight difference.

Polycyclic aromatic hydrocarbons analysis

Chemical reagents

All chemical reagents procured for the PAHs analysis were of high performance liquid chromatography grade; acetone, dichloromethane and hexane were obtained from Sigma Aldrich (Steinheim, Germany). The standard mixture of PAH was obtained from Augsburg, Germany.

PAHs extraction and purification in fish samples

The Wretling et al. (2010) method was used with minor modifications. Briefly, to the 10 g homogenized fish muscles per sample in a 250-mL round bottomed Erlenmeyer flask, 40 mL of dichloromethane (99.8% pure) extraction solvent was added and the flask was thoroughly sealed with aluminum foil for 30 min to prevent evaporation. To mixture in the flask, 5 g of anhydrous sodium sulphate was added. The content was shaken vigorously in a reciprocating shaker for 15 min. The content was further homogenized using auto vertex mixer for 5 min and allowed to settle for 10 min to ensure that the layers separate. The liquid layer was filtered to another 250-mL round bottomed Erlenmeyer flask by passing it through a separatory funnel packed with a glass wool to a height of 2 cm. The flask was washed with methanol/water (20 ml; 4:1 v/v) and content added to the funnel. The extract was then dehydrated by passing it through anhydrous sodium sulphate in a separatory funnel packed with florisil to a height of 5 cm and conditioned with 20 mL dichloromethane. The flask was rinsed with 10 mL dichloromethane and rinsing added to the separatory funnel. The clean and clear aqueous content was decanted into a 50-mL spherical flask and concentrated to 1 mL using a rotary evaporator at 35°C. The extracts were further purified as described by Mottier et al. (2000). A chromatographic column (1 cm internal diameter, id) was plugged with glass wool at the base. Activated silica gel was put in the column to a height of 5 cm. Additional 2 g sodium sulphate was added to the column. The packed column was then conditioned with 10 mL dichloromethane, thereafter 1 mL concentrate was loaded into the column and eluted with 20 mL dichloromethane. The eluate was transferred to a conical flask and concentrated to 1 mL in a vacuum rotary evaporator at 35°C. The eluate was further concentrated under nitrogen flow at 37°C to near dryness. To the concentrate, 1 mL of cyclohexane (containing 99.5% purity) was added and the content transferred into 2-mL amber glass vials with Teflon lined screw cap using pipettes for gas chromatography-mass spectrometry (GC-MS) analysis. To minimize PAHs volatilization, vials were kept in deep freezer at -20°C prior to analysis.

Chromatographic analysis of fish samples

Samples were analyzed with an Agilent 6890N gas chromatography instrument coupled with a 5975-mass spectrometry (Agilent Technologies, Santa Clara, CA, USA) following standard procedures (Lee et al., 2015). To separate the compounds, 1 µL sample solution was injected in the pulsed split-less mode onto a HP-5MS column with 30 m × 0.25 mm id. The column was programed as follows: injector temperature; 300°C, oven temperature at 80°C and held for 1 min, 245°C (6°C/min), 270°C (30°C/min) and held for 10 min, and 310°C held for 10 min. Other operating conditions were pulse pressure 45 psi, pulse time 0.9 min, purge flow 50 mL, purse time 1 min. Helium gas at constant flow rate of 1.1 mL/min was used as the carrier gas. The mass spectrometry was operated in electron impact ionization mode at 70eV with a solvent delay of 3.75 min. Identification and quantification of individual PAHs was confirmed by comparing the mass spectra and the peak retention time with those of the PAHs standards in the equivalent conditions. A retention time match of ±1% was considered for confirmation (Samuel et al., 2010).

Identification and quantitation of PAHs in fish samples

External standard was used for the identification and quantitation of PAHs. A stock solution of 20 µL/mL obtained from Sigma Aldrich (Steinheim, Germany) was prepared by diluting 0.2 mL of a 1000 µg/mL PAHs standard to 10 mL with cyclohexane. 1 µg/mL standard working solution was prepared by diluting 0.5 mL of a 20 µg/mL PAHs standard stock solution to 10 mL with cyclohexane. PAHs in the samples were identified and quantified by a combination of a retention time and mass spectral match against the calibration standards. Sample peak areas were compared to peak areas of the standard. A minimum of three concentration levels of the standards ranging from 0.1 to 5 ppm were injected into the GC-MS and calibration curve for each standard was obtained by plotting peak area against concentration of the standards. Each PAH in the sample was then quantified using the formula;

$$Cs\ (\mu g/kg) = \frac{(Ac \times 100)}{Ws \times R} \times 100$$

In which, *Cs* is the concentration of PAH in the sample in µg/kg, *Ac* is the concentration (ng/ml) relative to the highest part in the injection volume (µl), *Ws* is the mass (in grams) of the sample extracted and *R* is the recovery. For control purpose, solvent blanks were included in every run during the analysis. Arithmetic means and standard deviations were computed from quantifiable samples only. A computer program XLSTAT (version 7.5.2) was used for the calculation.

Percent recoveries (%R)

To determine the average percent recoveries, a set of three samples (30 g) of the homogenized fish muscles were spiked with 50 µl mixture of the 16 PAH standards of concentration ranging from 2 to 10 µg/L. Another set of three samples (30 g) were set as controls. Both the test and the control samples were allowed to stand for 24 h to allow absorption of the added PAHs into the matrix. The two sets of the samples were then taken through the same analytical procedures. The percent recoveries were calculated using the formula;

$$Recovery\ (\%R) = \frac{Cs-Cu}{\mathrm{Cn}} x100$$

In which, Cs is the concentration of PAHs in the test sample, Cu is the concentration of PAHs in the control sample, Cn is the (nominal) theoretical concentration increase that results from spiking the samples. The mean percent recoveries obtained were within the range (70 to 130%) with an average of 89.28% (Table 1). These values were relatively quantitative and were therefore used without any correction. The limits of detection (LODs) and limits of quantification (LOQs) ranged from 0.01 to 1.7 µg/kg and 0.02 to 5 µg/kg, respectively (Table 1).

Statistical analysis

Data obtained from the study was analyzed using R (R Core Team, 2018). A two-way analysis of variance was used to test the difference in proximate nutritional values and concentration levels of PAHS in fish samples smoked using *chorkor* and pit ovens with fresh samples as reference. Tukey's Honest Significant Difference Test was done where means of the two groups under comparison were significantly different. Level of significance was measured at $P \leq 0.05$.

Table 1. Retention time, limits of detection (LOD) and quantitation (LOQ), and percent recoveries (%R) of the 16 priority PAHs.

PAH compounds	Retention time (min)	LOD in µg/kg	LOQ in µg/kg	R^2	% Recovery
Naphthene	8.46	0.06	0.02	0.9993	88.70
Acenaphthylene	13.00	0.02	0.06	0.9995	82.40
Acenaphthene	13.26	0.02	0.06	0.9996	91.30
Fluorene	14.49	0.02	0.06	0.9994	90.80
Phenanthrene	17.14	0.03	0.09	0.9995	91.80
Anthraces	17.22	0.02	0.06	0.9998	86.10
Fluoranthene	20.16	0.04	0.12	0.9993	80.80
Pyrene	20.49	0.04	0.12	0.9998	93.00
Benzo [a] anthracene	23.55	0.06	0.20	0.9997	91.70
Chrysene	24.00	0.06	0.20	0.9993	89.60
Benzo [b] fluoranthene	26.30	0.01	0.30	0.9999	94.20
Benzo [k] fluoranthene	26.35	0.15	0.50	0.9996	93.40
Benzo [a] pyrene	27.18	0.15	0.50	0.9996	82.80
Benzo [g, h, i] perylene	30.06	0.75	2.50	0.9997	87.10
Dibenzo [a, h] anthracene	30.17	0.90	2.70	0.9991	94.00
Indenol [1,2,3-cd] pyrene	30.55	1.70	5.00	0.9994	90.80

RESULTS AND DISCUSSION

Proximate chemical composition of *M. caschive* and *O. niloticus*

As expected, the mean moisture content in fresh *M. caschive* (56.69±1.01%) was significantly higher than pit (15.3±0.57%), and *chorkor* (10.0±0.83%) smoked fish, P<0.05. In regards to the technologies, *chorkor* oven significantly reduced moisture content in *M. caschive* (10.0±0.83%) more than pit (15.3±0.57%), P<0.05. Similarly, the mean moisture content in fresh *O. niloticus* (64.34± 0.66%) was significantly higher than pit (17.23±0.42%) and *chorkor* (15.08±0.48%) smoked fish while *chorkor* oven reduced moisture to 15.08±0.48%, pit reduced moisture to 17.23±0.42%. This finding showed a significant reduction of moisture content in fish smoked using *chorkor* than pit. Indeed, the ultimate aim of fish smoking is to reduce moisture content that supports bacterial activities, oxidation or rancidity leading to spoilage (Adeyeye and Oyewole, 2016). Studies noted that high moisture provides conducive environment for spoilage microorganisms to thrive (Muhame et al., 2020). Heat application during smoking thus, breaks the hydrogen bond resulting to free molecules which eventually evaporate on the surface of fish (Akintola, 2015). Excessive loss of moisture leads to decreased water activity in fish tissues (Olukayode and Paulina, 2017). Effective method of fish preservation should thus reduce moisture to the recommended level of less than 20% depending on the purpose and the desired products (FAO, 2016).

Concerning performance of the two technologies; *chorkor* effectively reduced moisture to 10 and 15%, 15 and 17% for *M. caschive* and *O. niloticus* smoked using pit, respectively. In line with other studies (Adeyeye et al., 2015; Omodara et al., 2016; Katola and Kapute, 2017; Olukayode and Paulina, 2017), *chorkor* is an efficient technology for reducing moisture in fish muscle. Effective removal of moisture is attributed to heat concentration linked with the construction materials and enclosed characteristics of *chorkor* oven. While moisture content of *chorkor* smoked fish products in this study was within the level considered acceptable for smoked fish to inhibit both bacterial and fungal growth (Msusa et al., 2017), relatively higher values recorded in fish smoked using pit facility signify susceptibility of products to microbial spoilage particularly during storage.

Regarding nutritional quality, fresh *M. caschive* contained; 22.20±0.40% crude protein, 18.23±0.30% fat content and 9.13±0.14% ash. *M. caschive* smoked using pit kiln had 22.21±0.50% crude protein, 18.30±0.31% fats and 9.13±0.14% ash content. *M. caschive* smoked using *chorkor* had; 22.23±0.77% crude protein, 18.44±0.12% fat content and 9.24±0.08% ash. The results revealed c*horkor* oven concentrated the nutrients more than pit kiln. Equally, fresh *O. niloticus* contained; 24.24±0.27% crude protein, 8.87±0.34% fat and 9.28±0.22% ash content. *O. niloticus* smoked using pit had 24.24±0.25% crude proteins, 8.90±0.35% fat, 9.30±0.23% ash and *O. niloticus* smoked using *chorkor* contained; 24.30±0.27% crude protein, 8.92±0.35% fat and 9.35±0.21% ash. Similarly, *chorkor* concentrated nutrients more than pit technology. Results of this study supports the findings of other scholars regarding nutrient retention (Akintola, 2015; Abraha et al., 2017). However, the slight difference in the nutritional values of the two smoking facilities could be attributed to higher heat retention capacity of the

Table 2. Types of PAHs detected and quantified in smoked fish (µg/kg wet weight).

PAHs type	*M. caschive* (X±SD)			*O. niloticus* (X±SD)		
	Fresh	Pit	*Chorkor*	Fresh	Pit	*Chorkor*
Acenaphthene	n.d	0.82±0.00	n.d	n.d	n.d	n.d
Anthracene	n.d	2.31±0.00	n.d	n.d	n.d	n.d
Benzo [a] anthracene	n.d	7.83±0.00	n.d	n.d	n.d	n.d
Benzo [a] pyrene	n.d	n.d	n.d	n.d	n.d	n.d
Benzo [b] fluoranthene	n.d	n.d	n.d	n.d	n.d	n.d
Chrysene	n.d	4.65±0.00	n.d	n.d	n.d	n.d
Fluorene	3.75±0.10	3.83±0.10	3.64±0.11	n.d	3.81±0.09	n.d
Phenanthrene	n.d	2.69±0.00	n.d	n.d	n.d	n.d
Naphthalene	2.86 ±1.22	5.86±4.16	5.53±1.90	2.37±0.62	3.64±1.59	2.82±2.10
ΣPAHs	6.61	27.98	9.17	2.37	7.45	2.82
ΣPAH4	0.00	12.47	0.00	0.00	0.00	0.00

n=6 per treatment, n.d=not detected; **Σ**PAH4 is the sum of Benzo [a] anthracene, Benzo [b] fluoranthene Benzo [a] pyrene) and Chrysene.

construction materials used in *chorkor* oven that efficiently break down hydrogen bond in water molecules leading to water evaporation from the surface of fish.

PAHs levels in *M. caschive* and *O. niloticus* smoked using pit and *chorkor* kilns

During the study, seven types of PAHs consisting of chrysene, benzo [a] anthracene, anthracene, phenanthrene, fluorene, acenaphthene and naphthalene were recorded from the smoked fish samples (Table 2). The PAHs types recorded in pit and *chorkor* smoked fish products were mostly low and medium molecular weight (LMW, e.g. naphthalene, 2-ringed; anthracene, fluorene, acenaphthene and phenanthrene, 3-ringed and MMW, benzo [a] anthracene and chrysene; 4- ringed) compounds. In terms of abundance, naphthalene was the most occurring PAH followed by fluorene, benzo [a] anthracene, chrysene, phenanthrene, anthracene and acenaphthene. Fluorene and naphthalene were detected in fresh samples of *M. caschive* (Table 2). The high molecular weight (HMW, benzo [b] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene and dibenzo [a, h] anthracene, 5-ringed; Benzo [g, h, i] perylene and indenol [1, 2, 3-c, d] pyrene, 6-ringed) compounds were not detected and quantified in all the samples analyzed. As such, the ΣPAH4 (benzo [a] anthracene, chrysene, benzo [b] fluoranthene, benzo [a] pyrene) was employed to determine the carcinogenic potency of PAHs measured in the smoked fish samples.

The concentration levels of each type of PAH in smoked samples of *M. caschive* and *O. niloticus* are presented in Table 2. The concentration levels of fluorene (3.75±0.10 µg/kg) and naphthalene (2.86 ±1.22 µg/kg) measured in fresh samples of *M. caschive* were significantly lower than those recorded in smoked samples. Pit smoked *M. caschive* had higher concentrations of fluorene (3.83±0.10 µg/kg) and naphthalene (5.86±4.16 µg/kg) than *chorkor* smoked samples (n.d and 5.53±1.90 µg/kg), $P<0.05$. Besides, the sum of PAH4 in pit smoked *M. caschive* (12.47 µg/kg) exceeded the maximum limit set by the European Union regulation (Table 2). Regarding *O. niloticus*, naphthalene was the only PAHs detected in fresh samples with a concentration level of 2.37±0.62 µg/kg. About the effect of smoking facilities, *chorkor* smoked *O. niloticus* had significantly lower naphthalene concentration (2.82±2.10 µg/kg) than pit smoked products (3.64±1.59 µg/kg), $P<0.05$. Fluorene (3.81±0.09 µg/kg) was only detected in pit smoked *O. niloticus* but not in the fresh and *chorkor* smoked fish samples.

Findings on the concentration levels of PAHs revealed that fluorene and naphthalene quantified in fresh fish samples were significantly lower than that recorded in smoked fish products. This is in conformity with reports that naturally, fish and aquatic invertebrates contain small and sometimes undetected quantity of PAHs absorbed from aquatic environment (Ongwech et al., 2013; Yusuf et al., 2015). Low PAHs measured in fresh samples could be due to degradation of compounds into other components during metabolic processes in fish body. Most of the PAHs (about 85%) were below the limit of detection in the fresh samples. This shows that, PAHs recorded in smoked fish samples were solely attributed to smoking processes as observed by earlier studies (Olabemiwo et al., 2013; Forsberg et al., 2013). Concerning samples, low molecular weight and medium molecular weight PAHs compounds were identified. This may be linked to the type of firewood used during the smoking process. Pagliuca et al. (2003) showed that smoke produced by hard woods usually generates high concentrations of less molecular weight PAHs. Although the individual PAHs of less molecular weights were

detected in quantifiable amount, their levels were much lower than the sum of PAH4 (chrysene and benzo *[a]* anthracene). This could be attributed to the fact that, medium molecular weight compounds (e.g. benzo *[a]* anthracene, pyrene, chrysene and fluoranthene; 4-ringed) and high molecular weight PAHs (e.g. benzo *[a]* pyrene, benzo *[b]* fluoranthene, benzo *[k]* fluoranthene and dibenzo *[a,h]* anthracene; 5-ringed; benzo *[g,h,i]* perylene and indeno *[1,2,3-c,d]* pyrene; 6-ringed) compounds are more resistant to degradation both in aquatic organisms including fish and the environment. Similar observation was made by Anyakora and Coker (2007), who assessed PAHs content in four fish species from the Niger Delta. Their study attributed the difference to the fact that fish samples used were procured in an environment heavily polluted by petroleum products. Linda et al. (2011) noted higher levels of HMW than LMW PAHs when they categorized PAHs in smoked fish products from Ghana. They accredited the difference to residues of former combustion processes that might have occurred in the facility. Ongwech et al. (2013) also reported higher concentration levels of HMW than LMW PAHs when they investigated PAHs in smoked *Lates niloticus* collected in some markets of Gulu District, Uganda. They endorsed such a difference to exposure of fish markets to heavy air pollution from automobiles as markets are situated along Gulu-Kampala highway. Ongwech et al. (2013) further explained the higher levels of HMW compounds in relation to the mechanism of PAHS formation in that, during extended smoking, chances are that LMW PAHs formed are subsequently converted to HWM compounds through addition of combustion products from continued wood pyrolysis. Likewise, further burning of aromatic hydrocarbons' residues may lead to formation of additional HMW PAHs and subsequently increase their concentrations in smoked fish products.

In this study, fish species smoked using pit kiln have higher ΣPAH4 concentration levels than *chorkor* smoked fish samples. This difference could be explained in relation to the design and standardization of the smoking facility and processes (time of fish exposure to the smoke heat and regulation of the facility's temperature). C*horkor* oven has standard structural design and materials; perforated iron sheet, wood trays, enclosed system that will help in the control and normalization of the smoking process (oxygen and temperature). Besides, the difference could be associated with the time spent smoking; 8-12 h in a *chorkor* oven and 18-24 h in pit kiln. Excessive burning of firewood due to free air supply in pit facility could have resulted into increase in temperature that consequently led to variation in the rate of fat exudation. High rate of fat exudation may lead to modification of fish surface due to the presence of oils (Linda et al., 2011). The oily surface allows deposition of PAHs and other smoke chemicals on fish surface with subsequent penetration into fish muscles (Lee et al., 2019). Additionally, difference in fat exudation created by elevated temperature may have increased the rate of PAHs deposition and penetration in the muscles of pit smoked fish.

The presence of individual PAHs in higher levels in pit smoked fish samples on the other hand could be related to excessive heat treatment and proximity of fish products to heat that might have increased the penetration of smoke chemicals into fish flesh (Forsberg et al., 2013). However, presence of low molecular weight individual PAHs in higher level could be as a result of average wood combustion temperature (60-80°C) during the smoking process that indeed, could not degrade PAHs of higher molecular weight (Yusuf et al., 2015; Slámová et al*.,* 2017).

It also suggests that the wood type; *Acacia seyal* used for smoking fish may probably contain smoke chemicals of low and medium molecular weight (LMW, 3 and MMW, 4-ringed PAHs). Besides, the presence of low molecular weight PAHs in fresh samples analyzed could be due to the occurrence of the compounds in the environment as a result of anthropogenic processes including forest burnings which could have been washed into water bodies and finally got their ways to fish. In regards to the use of sum of PAH4 as indicator for carcinogenicity, *chorkor* smoking can be adopted as a safer processing technology than pit kiln. Although the results of the current study revealed, smoked fish samples from the two technologies could be deemed safe for human consumption, chances are that consumption of pit smoked fish may pose health risk due to cumulative effect. This therefore, calls for the need to regularly monitor the concentration levels of PAHs especially the higher molecular weight (HMW, PAHs) compounds in smoked fish products of South Sudan.

Conclusion

The effect of smoking ovens on nutritional quality and concentration levels of PAHs in smoked *M. caschive and O. niloticus* were investigated and the study revealed, both smoking kilns retained the nutritional values of smoked fish. However, *chorkor* oven concentrated the chemical components of fish; protein, fats and ash more than pit resulting to better nutrients retention. The structural design of *chorkor* oven and standardization of its smoking process significantly reduced the rate of PAHs deposition, accumulation and migration into fish muscles. As such, fish smoked using *chorkor* oven were associated with less PAHs than pit smoked fish products. The present study recommends *chorkor* oven acceptance and utilization for artisanal fisheries of South Sudan. However, regular monitoring of PAHs concentration levels; tree species survey and profile of smoke to minimize use of species with high risk of PAHs for smoking fish should be undertaken.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Characterisation and visualisation of foam quality attributes such as foamability, foam stability and foam structure of coffee brews, whole uht milk and coffee-based beverages

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Consumers value foam as a key quality feature of coffee and coffee-based beverages. However, perceptions of foam quality vary from country to country and even from consumer to consumer. This study provided measurable parameters which can be used to tailor products to consumers' foam preferences. The analytical techniques described in this study enable the foam of coffee and coffee-milk beverages to be visualized and quantified in terms of volume and structure. It is intended to serve as a basis to quantify consumers' subjective expectations of different coffee-based products, in terms of appearance. The aim of the present study was to develop an analytical methodology for the objective assessment of customer-specific requirements. For this purpose, the parameters foamability and foam stability were assessed by means of image analysis, while changes in structure were measured with a Dynamic Foam Analyzer. All measurements were carried out in triplicate with an overall RSD of ≥7%. The proposed procedure allowed normally subjective quality features of a variety of foams in different coffee species and milk types to be objectively characterized. This knowledge will enable further investigation into tailoring the scientifically assessed foam properties to subjective sensory characteristics perceived by the consumer, such as smoothness, fineness, body, thus helping to better satisfy rapidly evolving coffee consumer trends.

Key words: Foamability, foam stability, foam structure, bubble size distribution, coffee crema, coffee-based beverages.

INTRODUCTION

Combined coffee and milk drinks are rarely studied and analysed together, although it is known how different they

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are (e.g. milk is alkaline and coffee is acidic; pH 4.5-5.0). The interaction between milk and coffee in the formation of milk foam is rarely mentioned in the literature, in contrast to the amount of knowledge on (black) coffee and milk foam. Many studies have already been carried out on the subject of coffee and milk foam (Hatakeyama et al., 2019), but mainly as separate investigations. A comprehensive review on coffee foam was published by Ishwarya and Nisha (2020). Analysis of the mechanism of foamability revealed that Maillard reaction products such as melanoidins, formed during the roasting of coffee beans, are responsible for the foaming of espresso coffee. Furthermore, two additional reactions that occurred in the espresso brewing method can be indicated as a source of carbon dioxide. Both reactions derived from bicarbonate ions contained in the water, the second most important ingredient for brewing coffee. In particular, carbon dioxide is formed from bicarbonate thermal decomposition and from bicarbonate neutralization by natural coffee acids (Illy and Navarini, 2011). Researchers have confirmed that polysaccharides such as galactomannans and protein complexes have surface and rheological characteristics that improve foam stability (Nuñes and Coimbra, 1998). In another study, these authors have reported that the foaming capacity and stability of espresso coffee increases with the degree of roasting, as well as in the presence of galactomannan, arabinogalactan, and proteins. These attributes do not vary according to the origin of the coffee (Nuñes et al., 1997).

Increasingly important in the market for milk-based coffee drinks is the quality of the milk foam. Milk fat and milk proteins are crucial for the production of stable milk foams with the desired texture and stability (Huppertz, 2014). Milk proteins stabilise the air bubbles in milk foams. However, foam stability decreases over time as liquid flows out of the foam, causing air bubbles to come into close contact and fuse together. This leads to ever larger air bubbles which eventually collapse. Milk fat also destabilises the foam, but is desirable for its contribution to taste. The foaming and stability of milk foam depend on the composition of the milk used and are influenced by the physicochemical properties of whey protein (Khezri et al., 2017). The production of stable milk foams therefore involves a complicated process of balancing the desired foaming properties of the milk proteins and the destabilising milk fat required for taste (Borcherding, 2004; Borcherding et al., 2008; Kamath et al., 2008; Borcherding et al., 2009; Marinova et al., 2009; Huppertz, 2010).

A study by Hatakeyama et al. (2019) evaluated the sensory characteristics of foamed milk. These authors have described that in their study foamed milk with small bubble size produced high sensory scores for fineness, smoothness, and elasticity. The stability of foam presented a challenge, particularly due to the fragile nature of gas bubbles. Generally, foams are destabilized by liquid drainage, the coalescence of bubbles, and the disproportionation of bubbles (Silva et al., 2008). Obviously, these properties depend on the composition of the gas and liquid phases. For cappuccino-type beverages, for example, the foam should be stable for the amount of time typically required to consume the drink (Huppertz, 2010). Bubble size is also important for foaming properties in general. Although bubbles are small immediately after foaming, they coalesce and become large with time, and finally burst. A small bubble size in a café latte contributed to the sharp visual contrast between the foamed milk and the coffee (Borcherding et al., 2008).

Chemical methods of foaming have gained importance in recent times. Compared with physical foaming, in chemical foaming, the desired bubble characteristics such as bubble size, bubble size distribution, and bubble generation rate are achieved with considerable ease (Pugh, 2016a). It has been reported that in order to obtain a better foam in cappuccino coffee, foaming creamers such as food-grade acidulants, sodium carbonate, or sodium bicarbonate could be added as chemical foaming agents (Agbo et al., 1998).

Several techniques are available for the measurement and characterization of foams based on the bubble size distribution, foam height, rheological characteristics, interfacial properties, and gas diffusion rate (Pugh, 2016b). Using a Dynamic Foam Analyzer (DFA), bubble count, bubble area, and foam height have been investigated in foamed milk (Hatakeyama et al., 2019). In this investigation foam height was defined as the total height of liquid and foam. Yekeen et al. (2016) employed DFA 100 in 2016 to estimate the effect of silica nanoparticles on sodium dodecyl sulfate (SDS) foam stability in the presence of oil. Apart from bubble size distribution, foam volume, foam height, and coalescence of bubbles were also examined from images captured (Yekeen et al., 2017). Recently, Deotale et al. (2020) used coffee oil as a natural surfactant to stabilize coffee foam. They employed DFA for studying bubble characterization using coffee oil in comparison with non-ionic surfactants. Other approaches to determine bubble size are electroresistivity (Barigou and Greaves, 1991), optic (Ghiassi et al., 2012; Upadhyay et al., 2012; Kulkarni et al., 2004) and acoustic techniques (Prabhukumar et al. 1996; Anguelova 1997; Duraiswami et al. 1998; Wu and Chahine 2010; Czerski, 2012; Leighton, 2012).

In this study, a commercial Dynamic Foam Analyzer using specific image-processing software was employed for the characterization of the volumetric foam properties of the foam produced. This set-up was also applied to determine foam structure as a function of time for different foams produced in coffee extractions, milk only and coffee-milk combinations. Additionally, a relatively inexpensive photographic setup (compared to the cost of the DFA equipment) was used to measure the actual foam decay.

MATERIALS AND METHODS

Coffee machine for milk foam preparation

A fully automatic Schaerer coffee machine of the type Coffee Soul (Schaerer Ltd., Switzerland) was used in this investigation. The following settings were adopted for the foaming of milk (Cup parameter): Duration (s) -40; Temperature (C°) - 65, Air-pump-speed (%) - 45; Air-start-temperature (C°) - 25; Air-stop-temperature (C°) - 40.

Coffee machine for coffee and coffee-milk foam preparation

A Turmix capsule machine, model TX 170 CITIZ NESPRESSO (Nespresso, Switzerland), was used to produce the coffee and coffee-milk samples.

Dynamic foam analyzer DFA 100

A Dynamic Foam Analyzer (DFA) 100 (KRÜSS GmbH, Borsteler Chaussee 85, Hamburg, Germany) was used to measure the foaming structure of the samples. The device consisted of an infrared LED line for improved detection, an external temperature sensor for direct measurement of the sample temperature and a glass column CY4572 (d = 40 mm). The software ADVANCE for DFA Version 1.11-03 combined control of the instrument with data processing functions.

High resolution camera

To measure the foamability and the true foam decay in milk and coffee-milk systems a Thorlabs DCC1645C high resolution USB2.0 CMOS camera (Thorlabs GmbH, Lübeck, Germany) was used, with a full-frame resolution of 1280 × 1024 and a colour sensor with a 50 mm fixed focal length lens.

Volluto coffee capsules

For the preparation of the coffee brews, commercial Volluto Nespresso capsules were used for all systems:
1. Strength 4 on the Nespresso scale
2. Medium roasted
3. Pure Arabicas from Brazil and Colombia
4. Medium grind

Whole milk UHT

Key figures of the EMI UHT milk with a fat content of 3.5% (measurements from Emmen Laboratory):

Pasteurisation temperature/time:	139°C/4 s
Homogenization pressure:	195/40 bar
Precise fat content:	3.45 g/100 g
Protein content:	3.47 g/100 g
Lactose content:	4.2 g/100 g
Viscosity:	28.0 mPas (5°C)

Sample volumes

The volume of the samples was chosen in accordance to Nespresso's suggestions. *https://www.realcoffee.com/cup-size-programming/*

1. Double espresso:	Approximately 80 ml
2. Latte macchiato:	40 ml coffee and 150 ml milk
3. Foamed milk:	120 ml

Patent blue solution (1 g/L)

100.00 mg patent blue was weighed into a 100 ml volumetric flask and filled up to the mark with deionised water.

Foaming procedures

Procedure for milk foaming

150 g whole milk (145 ml) cooled to approx. 4°C was weighed into a separate milk can and foamed. The foaming time was 30 s. The frothed milk was then poured into the cylindrical glass column of the DFA device for the measurements. The temperature just after foaming T_0 was 60° C (+/-2°).

Procedure for foaming coffee only

85 g of coffee extract was brewed directly into the cylindrical glass column of the DFA device. The temperature just after foaming T_0 was 65°C (+/-1°).

Procedure for foaming coffee-milk beverages

For the measurements of the coffee-milk samples, 40 g of coffee extract was first brewed directly into the cylindrical glass column of the DFA device, and then all the previously foamed milk was poured onto it for measurement. The temperature T_0 was 62° C (+/-1°).

Procedure for enhanced visualisation of visual contrast in samples containing milk

100 g of the cooled milk sample was weighed into a 200 ml beaker and 10 drops of the patent blue solution were added just before the start of the foaming experiment.

Foam characteristics

The measurement time for all analysis was 600 s. The foam in the glass cylinder was characterised at four time intervals (t_0, t_1 min, t_3 min and t_5 min). All measurements were in triplicate. The RSD was for all experiments ≥ 7%.

Foamability

The foamability ratio (FR) introduced here was used as a measure of foamability. The foamability was determined by setting the total height (liquid plus crema) at T_1 min after foaming in relation to the initial liquid height resulting in the FR. The arbitrary setting of the readout at T_1min was chosen as from this point on the phase boundaries are clearly visible. For the sake of simplicity, the liquid mass used was converted into height by assuming a density of 1g/cm^3 regardless of the nature of the sample.

$$Foamability\ Ratio\ (FR) = \left(\frac{Total\ Height\ (mm)}{Height\ of\ liquid\ \ before\ \ foaming\ \ (mm)}\right)$$

Table 1. Foamability ratios (FR)*.

Sample	Volume (ml)	Height of liquid before foaming (mm)	Total height T_1 min (mm)	FR (RSD)
Volluto coffee	85	70	94	1.34 (+/- 7%)
Whole milk	145	115	173	1.50 (+/- 3%)
Volluto coffee-	145 milk	150	207	1.38(+/- 3%)
Milk beverage	40 coffee			

*Measured in triplicate.

Table 2. Foam decay and foamability ratios* of the investigated beverages

Sample	Foam decay (FD) (T_1- T_3 min) (%)
Volluto coffee	25.0
Whole milk	14.6
Volluto coffee-whole milk beverage	12.2

*measured in triplicate (RSD ≥ 7%).

Foam stability

The true foam decay (FD) determinations in milk containing samples were carried out with the Thorlabs, high resolution camera. This simplified method measures the differences in foam heights (mm) immediately after foaming and change of foam height after 3 min. These measurements were determined visually from the photos taken.

Foam structure

The structure measurements lead to bubble count and size obtained from the analysis of CCD camera images of a defined area (1280 × 1240 pixels). Their statistical size distribution over time is depicted as histograms.

RESULTS AND DISCUSSION

Foamability

As shown in Table 1, the highest foamability ratio occurred in the milk samples, while the coffee and coffee-milk beverages investigated exhibited slightly lower values but a similar trend. The foamability ranged from 1.34 (+/- 7%) for coffee samples to 1.50 (+/- 3%) and 1.38 (+/- 3%) for whole milk and coffee-milk beverages respectively (Table 1).

The rather high RSD in the FR of the coffee samples can be explained by the fact that the upper foam threshold showed a very irregular and porous layer, making the determination of the actual height quite subjective (Figure 1, T_0). It can be said that the reading of this height strongly depended on the observer and the proposed FR should be taken as a semi-quantitative value. Further studies are needed to determine influences such as grinding, extraction, roasting degree, portion in coffee machine, percolation conditions, coffee species, and milk fat content on the resulting foam volume.

Foam stability measurements with enhanced foam-liquid interface visualisation

A simple method was adopted for testing foam stability without an expensive measuring device. This consisted in measuring the foam height at one minute and at three minutes (Table 2). Since the foam-liquid interface was apparently not yet fully formed or was not visible at T_0, the difference between the foam heights at T_1 and T_3 minutes was chosen to determine the foam stability. The ability to visualize a process within a multiple phase liquid is typically carried out using optical sensory. However, when a displacement study uses untransparent immiscible phases, identification of the phases in a specific location may be a challenge. The visual decrease of the foam as a function of time is shown in Figures 1-3. Although, the foam-liquid interface can be clearly seen by the eye (Figures 1 and 3) no satisfactory phase boundary differentiation between foam, liquid and air could be achieved by optic sensors.

In this investigation, the evaluation of foam stability was determined visually from the photos taken with an external camera over time. The stratification between the foam-air level and the foam-liquid level was measured manually directly from the pictures and provided the foam height in mm at a given time (Figures 1-3). In particular, the detection of the phase interfaces in milk samples caused some difficulty. As seen in Figure 2, shortly after foaming almost no interfaces were recognisable. Even after a time period of 1 and 3 min boundaries were very difficult to detect. To overcome this inconvenience, a methylene-blue solution was selected for the visualization of the two phases. When Figure 4 is compared with Figure 2, it can be seen that the phase interfaces can be differentiated much better using this technique. Without the addition of dye, the first few minutes of the

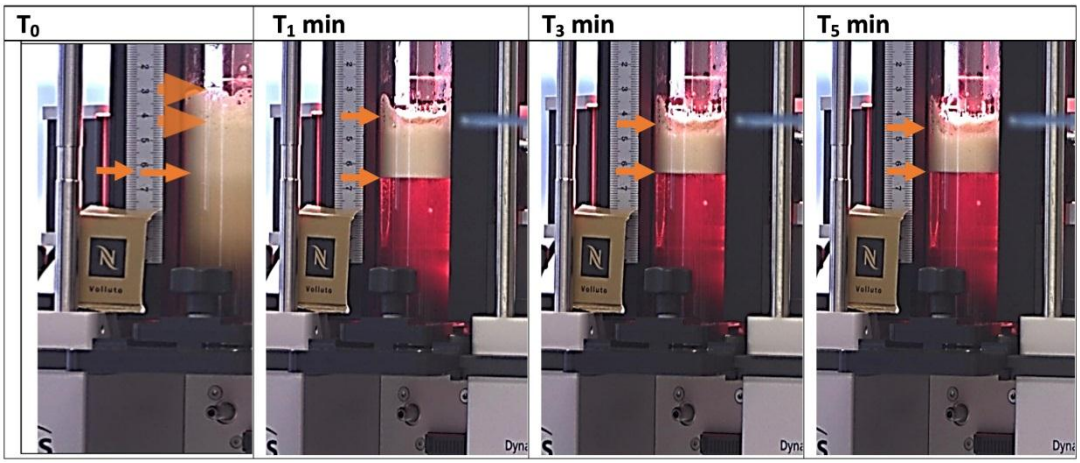

Figure 1. Foam decay over time of freshly prepared Volluto coffee.

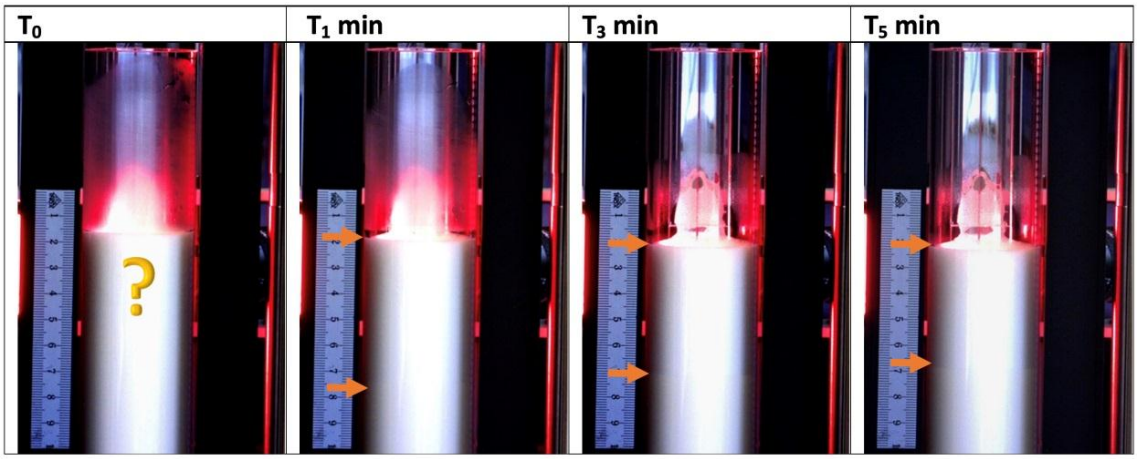

Figure 2. Foam decay over time of freshly foamed whole milk.

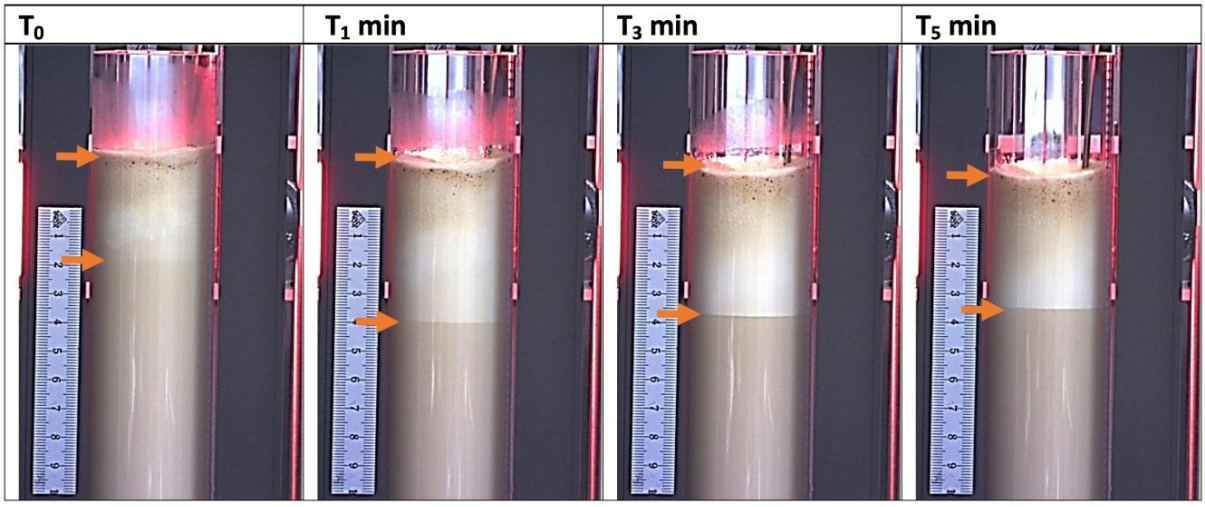

Figure 3. Foam decay over time of freshly prepared Volluto coffee-whole milk beverage.

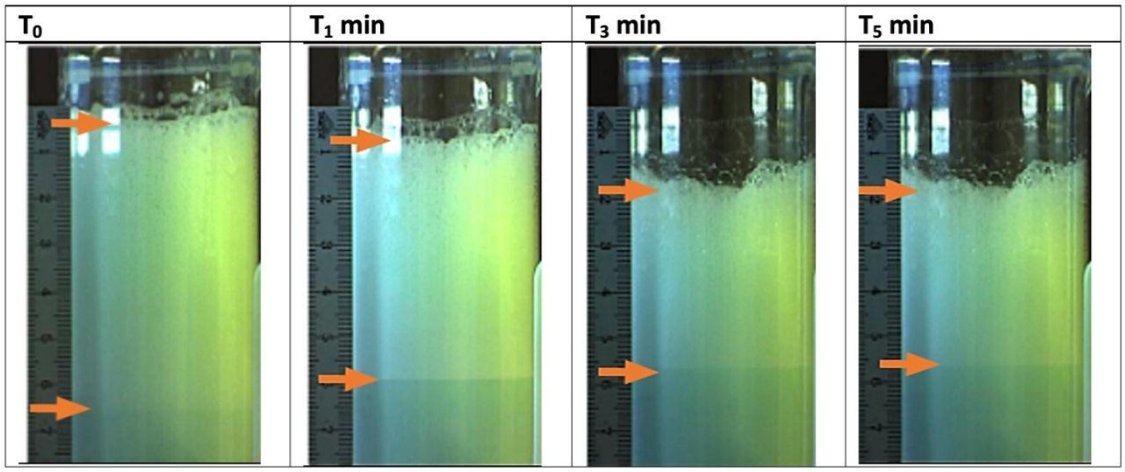

Figure 4. Enhanced visualization of the foam-liquid interface in freshly foamed milk.

measurements could not be evaluated. The readout of the value at T_1 min was arbitrarily chosen, as from this point on the foam-liquid interface was clearly visible, which was not the case immediately after the foaming process. The set-up used was validated and no influence of the colorant was found (n=3, RSD=≥7 %),

It is imperative that the influence of the additives on the foam properties should be re-evaluated after each modification or new configuration of the system. The experiments for enhanced visualization of the foam-liquid interface were performed in a separate glass cylinder in order not to interfere with the foam structure evaluations of the Krüss software. As seen in Table 2 the foam stability expressed in percentage of actual foam decay (FD) resulted in 25% for the coffee samples, 14.6% for the milk samples and 12.2% for the blend (RSD ≥7 %).

Foam structure

The instability of the foam, induced by drainage, coalescence and disproportionation, led to changes in the foam structure over time. Drainage is the liquid flow from a foam as a result of gravity and capillary forces. Because of drainage a foam becomes dryer and bubbles may become distorted. In that case foams change from spherical foams to polyeder foams. This could be observed for the coffee samples (Figure 5) but not for the coffee-milk beverages. Coalescence is the merging of two bubbles, leading to larger bubbles in the foam and a decrease in the number of bubbles. This process was seen in all the samples investigated. Disproportionation effects the distribution of the liquid and gas phase. Because of gas diffusion, larger bubbles grow at the expense of smaller bubbles and thus alter the appearance of the foam. The visual comparison of the foam structure given by the number and size of bubbles evaluated by the DFA system showed that the foam structure of the coffee samples was the finest (Figure 6). The texture of the whole milk samples was the coarsest, while the coffee-milk blends were in between the structures represented in Figures 5 and 7.

The foams produced with coffee samples (Figure 8) showed values for bubbles/mm^2 ranging from 450 shortly after foaming, decreasing to 175 bubbles/mm^2 after 5 minutes. In addition, some bubbles became larger, changing their shape from spherical to irregular. The foams produced with pure whole milk (Figure 9) showed initial values of 175 bubbles/mm^2 and ended at 60 bubbles/mm^2. This indicated that the foams already had coarser bubbles directly after the foam-generating process, with the number of bubbles decreasing significantly by the end of the measurement. This resulted in a coarser texture while the spherical shape was maintained over almost the entire measurement time. The coffee-milk beverage showed initial values of 230 to 175 bubbles/mm^2 at the end of the experiment (Figure 10). The initial shape can be defined as irregular, which towards the end of the measurement turns into a circular form. After an initial more or less accentuated stasis period, the bubble number/area decreases with time and follows the expected exponential decay. A high bubble count tended to lead to small bubble size. The exponential function of the bubble count/area versus time of the experiments was used to compare the decay of the foams of the different beverages investigated. The fitted exponential decrease of bubble count/mm ^{2} vs experimental time showed almost equal exponents, suggesting a similar decay rate for all the beverages investigated (Table 3).

Time-dependent analysis of bubble size distribution

Bubble size distributions were measured by the DFA technique and presented as histograms. The x-axis

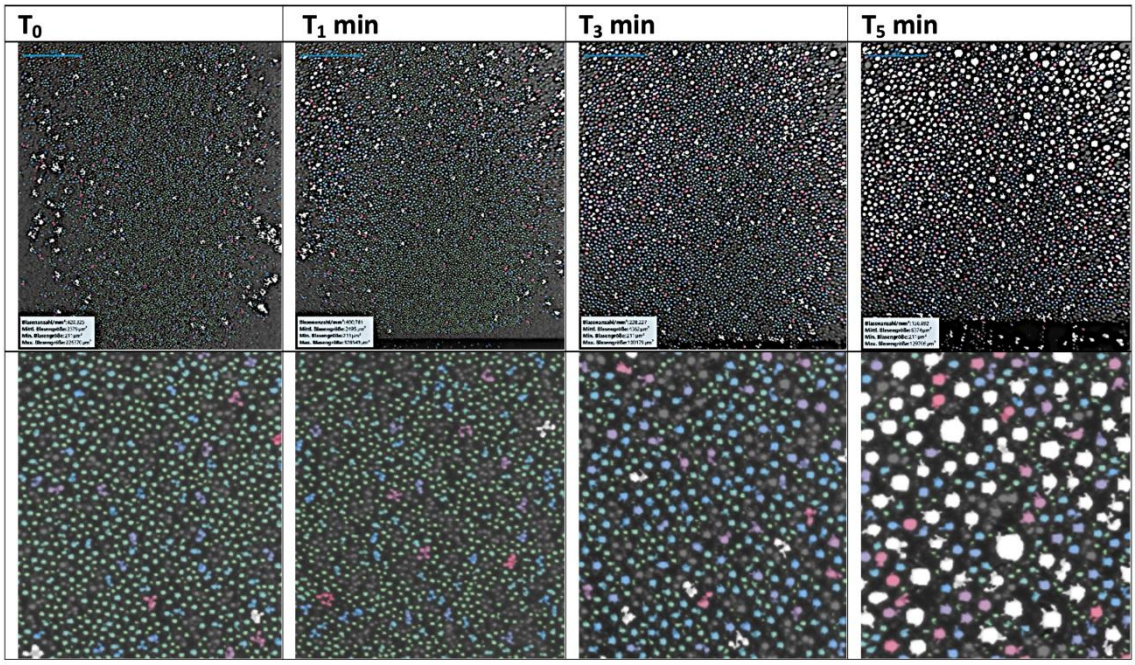

Figure 5. The upper half of the figure shows the appearance of the foam structure of freshly prepared Volluto coffee over time. The blue line of 2 mm length shows the absolute dimension of the bubbles. The lower half of the table shows a zoomed-in section of the upper image. The colour of the bubbles indicates their size (see Figure 11).

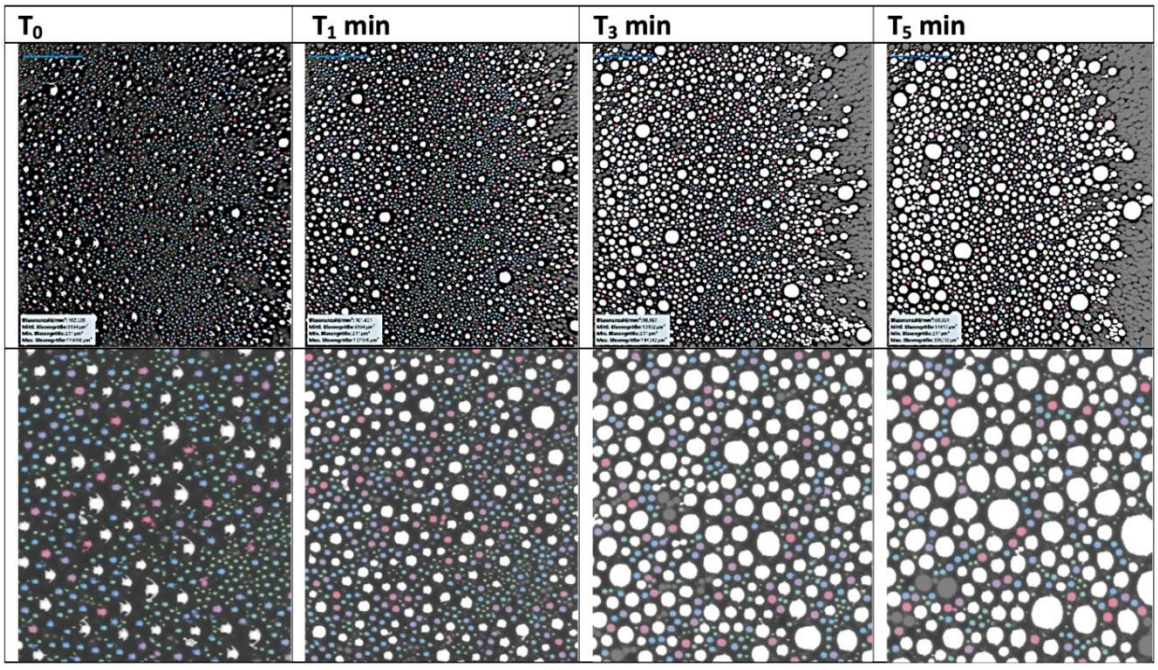

Figure 6. Appearance of the foam structure of freshly foamed whole milk over time.

represents the bubble area in μm^2 or pixels divided into classes as a colour-coded bar (Figure 11). The y-axis shows the count of the bubbles. It is automatically adjusted to the highest value by the software and cannot be scaled. For better visualisation, the highest values are shown magnified.

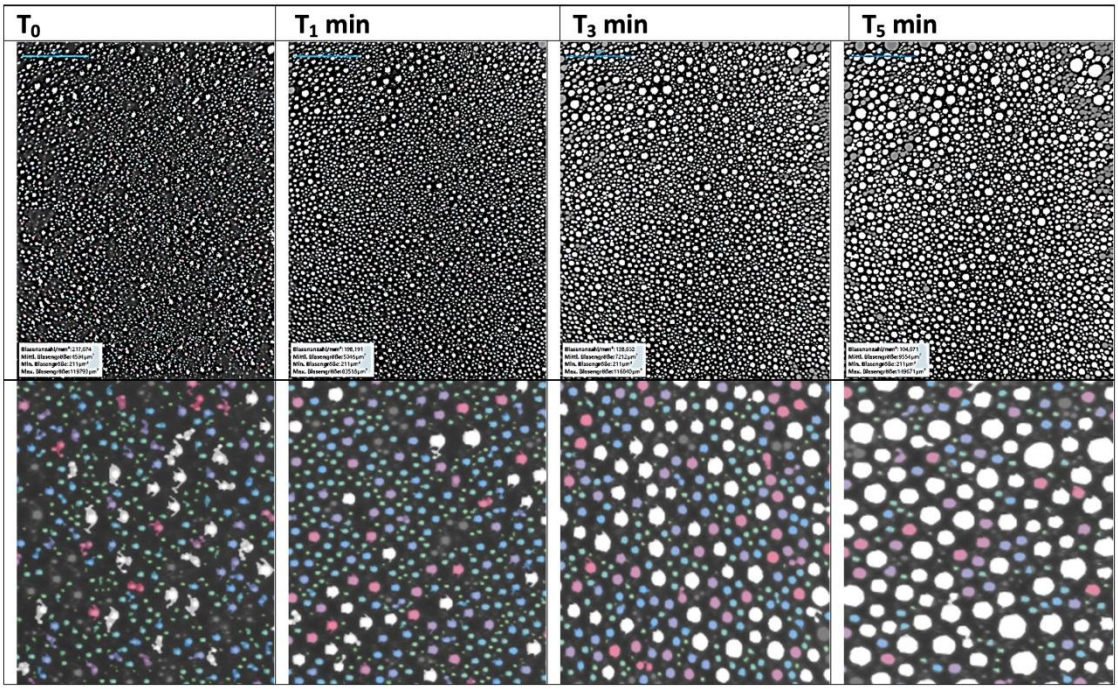

Figure 7. Appearance of the foam structure of freshly prepared coffee-milk beverage over time.

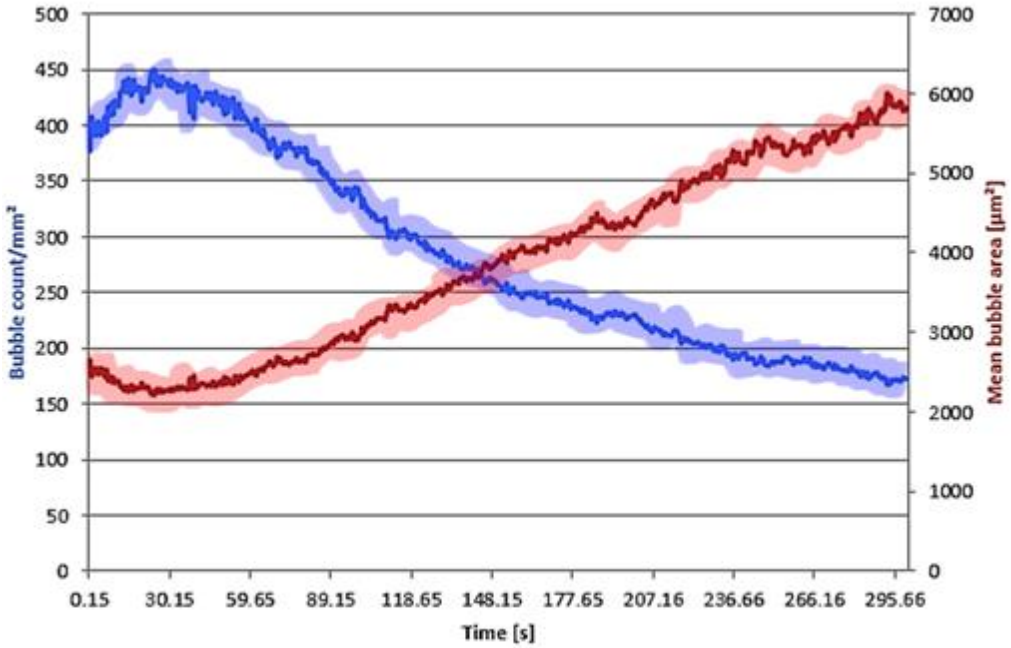

Figure 8. Bubble number and mean bubble size of freshly prepared Volluto coffee over time (n=3, RSD ≥7 %). The uncertainty bars have been set as lines with fill areas

For the coffee samples it can be observed that initially the bubble-size distribution was almost monodispersed, having relatively uniform small bubble sizes. All bubbles had a spherical shape (Figures 6 and 12) reaching moderate polydispersion and changed geometry at time T_5 min. A slight increase of bubble count with corresponding diminishing of the bubble size at around T_1 min could be observed. For the foam formation of milk

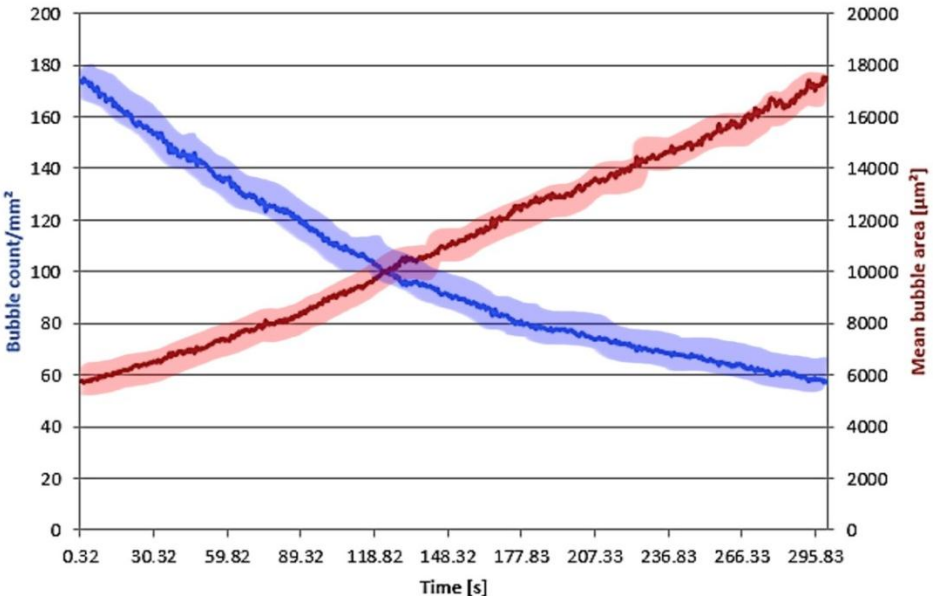

Figure 9. Bubble number and mean bubble size of freshly foamed whole milk over time (n=3, RSD ≥7 %). The uncertainty bars have been set as lines with fill areas.

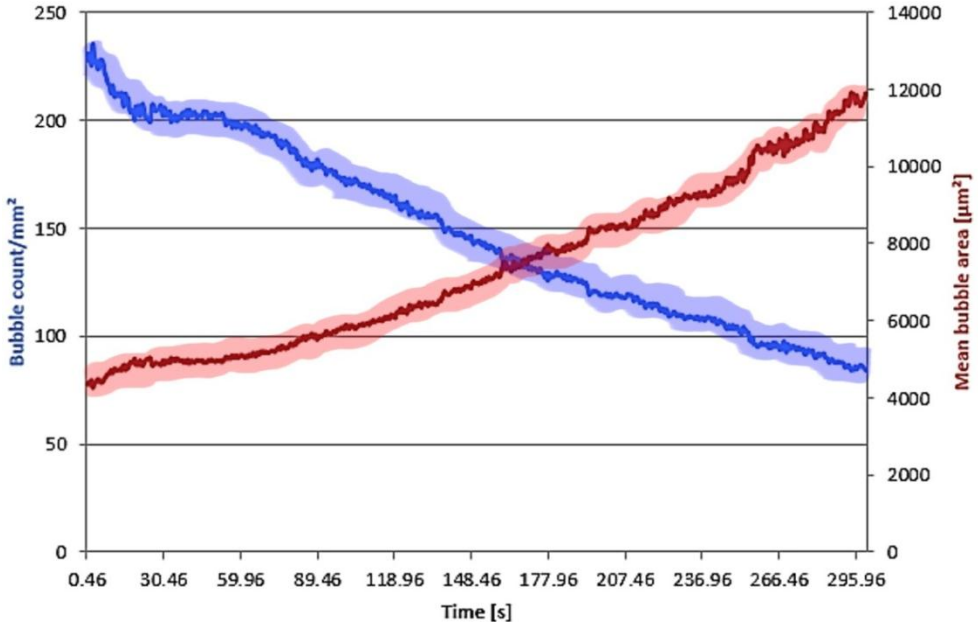

Figure 10. Bubble number and mean bubble size of freshly prepared coffee-milk beverage over time (n=3, RSD ≥7 %). The uncertainty bars have been set as lines with fill areas.

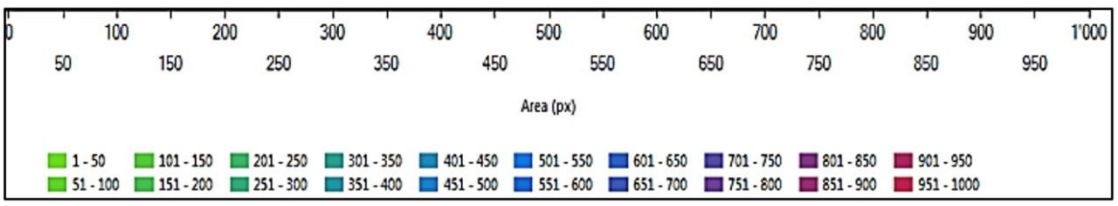

Figure 11. Legend of the x-axis: Bubble area at a given time; from left 1-50 µm^2 to right 1000 µm^2 or pixels.

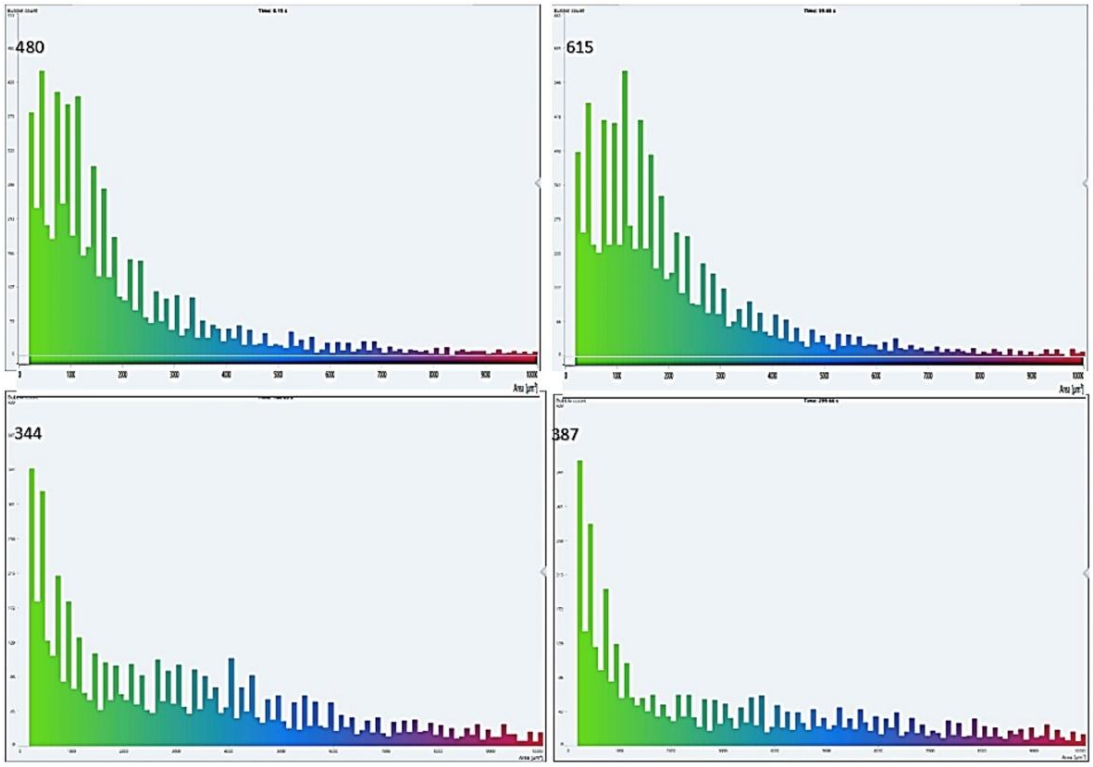

Figure 12. Upper histograms: freshly prepared Volluto coffee at T_0 min (left), T_1 min (right) Lower histograms: freshly prepared Volluto coffee at T_3 min (left), T_5 min (right).

Table 3. Exponential decay of bubble number/mm^2 vs experimental time.

Sample	Exponential function
Volluto coffee	$y = 465.8\ e^{-0.002x}$ $R^2 = 0.9759$
Whole milk	$y = 165.8\ e^{-0.002x}$ $R^2 = 0.9873$
Coffee-milk beverage	$y = 234.36\ e^{-0.002x}$ $R^2 = 0.9911$

samples, shown in Figure 13, the initial distribution was more polydispersed and this polydispersion was observed over the entire measurement period. The coffee-milk beverages showed a behavior between the two other beverages investigated. Moreover, the bubble size become progressively larger over time for all samples, while the bubble number reduced over time (Figure 14).

The foam analyzer used in this investigation played an important role in the time-dependent analysis of bubble size and distribution of bubbles using advanced image processing techniques. Although the apparatus is not that inexpensive, it permits to characterize foam properties easily and precisely. The beverages investigated, although different in total composition, presented almost the same decay profile in bubble count, with a similar increase of bubble size over the time of the experiment.

Conclusions

The analytical platform developed allows quantitative, and therefore objective determination of foamability, actual foam decay, foam structure and statistical bubble size distribution in coffee alone and in different coffee-based beverages. In this study, only one variety of coffee and one type of milk were selected as examples to show the feasibility of the analytical methods. These methods allow the foam properties to be visualized and quantified in terms of appearance, providing a basis for producing different coffee-based products that consistently satisfy consumer expectations.

It should be emphasized that the aim of the present study was not to investigate the foamability process itself nor to examine the different influences on it, but to develop an analytical methodology for the objective assessment of customer-specific requirements. The personalization of coffee-based products is of great importance against the background of rapid changes in a highly competitive market and a rapidly evolving technological environment. Only after subjective sensorial aspects of foam/crema and consumer preferences for other features, such as optical aspects, have been determined and correlated, using the objective analytical tools presented in this publication, can a desired product be created with a constant and satisfactory quality. This

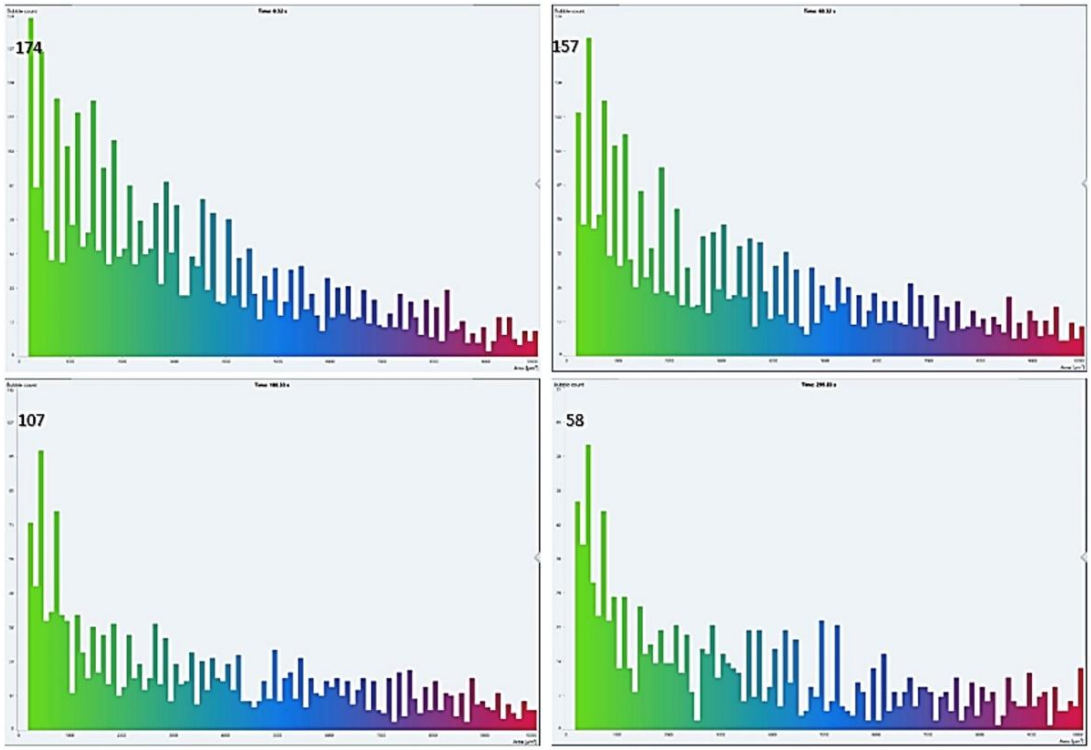

Figure 13. Upper histograms: freshly prepared fresh foamed milk at T_0 min (left), T_1 min (right) lower histograms: freshly foamed whole milk at T_3 min (left), T_5 min (right).

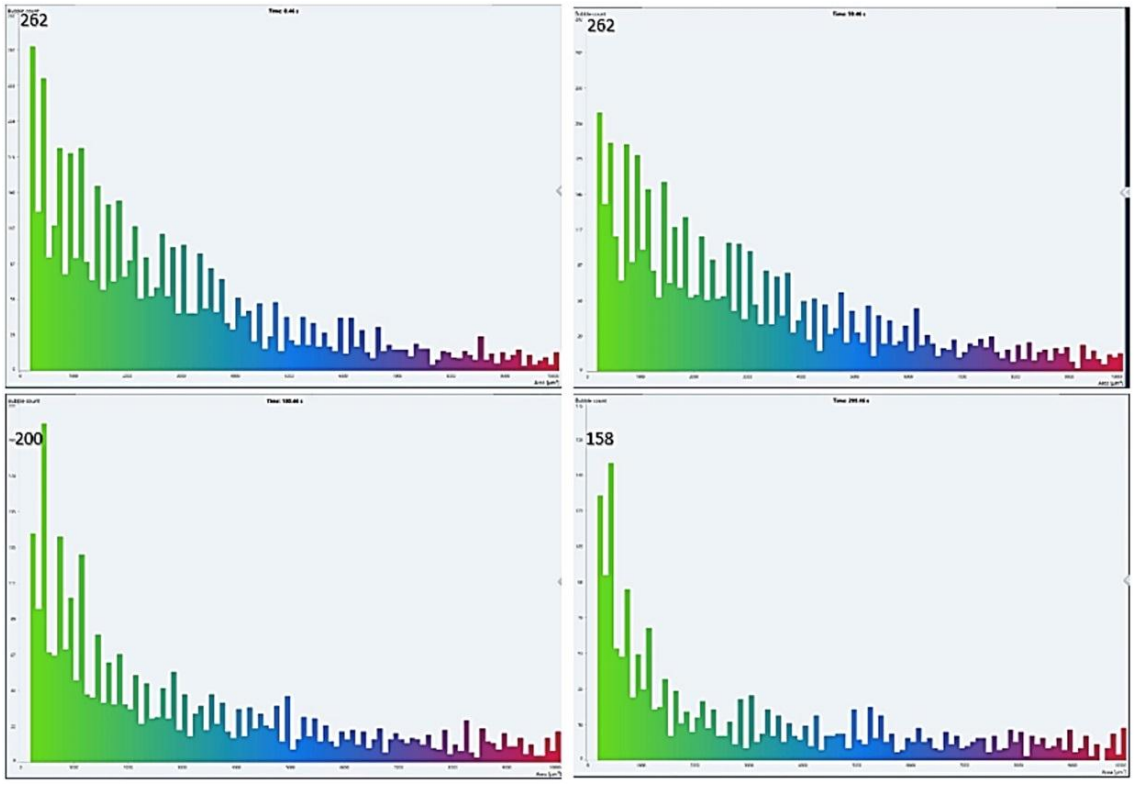

Figure 14. Upper histograms: freshly prepared coffee-milk beverage at T_0 min (left), T_1 min (right). Lower histograms: freshly prepared coffee-milk beverage at T_3 min (left), T_5 min (right).

will be the aim of further studies.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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