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Microwave effect on the physicochemical and emulsifying properties of crude whey

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ABSTRACT

The whey is a cheese effluent and a source of serious organic pollution seen its wealth in precious organic fractions (lactose and proteins) subsequently affecting the quality of the environment (freshwater ecosystem). The work we have done has aim of improving the emulsifying properties of crude whey (acid and soft) while changing their physical environment. For this, we monitored the one hand the variability of the apparent viscosity and temperature of crude whey (acid and soft) following treatment by microwave (2.45GHz, 800W) and secondly to estimate their emulsifying properties and the kinetic control of the size of the fat globules virgin olive oil and the interfacial surface of emulsions. The results showed that treatment with microwaves crude whey (acid and soft) has modified the physical properties (apparent viscosity); these vary according to experimental variables of microwave fixed (the frequency and the power restored) and also the residence time of the crude whey (acid and soft) and compared to witness, good emulsifying properties are marked for crude whey treated by microwave (2.45GHz, 800W / 45s for acid crude whey, 2.45GHz, 800W / 5s for soft crude whey).

Keywords: Microwave, viscosity, proteins, crude whey, emulsion.

INTRODUCTION

The whey is a cheese industrial co product; by its fermentable organic matters is the suitable factor of biological pollution of freshwater ecosystems; the release into the wild of about 50% of world production of whey engendered serious problems of pollution, 1000l whey with an average biochemical oxygen demand (BOD), 80000 ppm, equivalent to a pollutant to 400 people [18]. In Algeria, as in other countries in the world that rejection is about 85% of the milk transformed into cheese [19-20]; this vision, combined with environmental pressures increasingly strong exercised from 1960, prompted the dairy industry to be concerned about the valorization of waste whey for food, feed and possibly in the field of biotechnology [21]. The knowledge of primary and three-dimensional structures of the 6 major milk proteins and whey (caseins α_{s1} , α_{s2} , β and κ , β -lactoglobulin and α -lactalbumin) has helped to link these structures to physicochemical and functional behavior and predict the properties of an ingredient in a product depending on the conditions of preparation; whey proteins are increasingly used as texture ingredients in many intermediates products or ready to consume, more work is undertaken on the functional behavior and individual of caseinate and whey protein concentrates in aqueous solution and many journals have already established an inventory of hydration properties, the texture and surface [22-40]; emulsifying properties are part of these properties, one can then assess from the manufacture of the emulsions, these are dispersions of two immiscible liquids, one of which is in the form of dispersed droplets and the other as a dispersant continuous phase [21]. The study of the stability of emulsions is therefore essential to understand the parameters to obtain systems that meet the stability criteria defined a priori; various methods have been proposed to monitor the emulsion destabilizing including measures conductivity, turbidity, viscosity or ultrasound technical [71-40]. Several thermal and athermal techniques

are invented and validated to improve some qualities of ingredients and products manufactured for various domains [10-12]. In this sense, many topics were the subject of many published work for to determine the impact of the electromagnetic wave on the material used in the experiment [4-7,40]. Domestic microwave ovens have been widely in use from the beginning of the 1950; since the first study on the use of a microwave system for pasteurization of milk [14], several works on microwave milk treatment, mainly on microbiological aspects, have been reported [15]. A report in 1986 demonstrated that microwave energy is also suitable for accelerating organic reactions [16]. Unlike conventional heating techniques that used the convection or the conduction for supplying energy in a material, the microwave heating due to degradation of the heat energy of an electromagnetic wave within the material itself [9-8]. Heat treatments denature proteins but differently according to their nature [2]. The effects of denaturation can be numerous: changes in the solubility due at exposure of hydrophilic or hydrophilic peptide sites [3], modification of the power of water retention, modification of biological properties, the changing the action of proteolytic enzymes, changing the viscosity of the solutions, as well as the modification of allergenic potential sites. The viscosity of the continuous phase is a key parameter in emulsion stability; more it is increased more the size of the globules of the dispersed phase is lower and the aging time is long [13]. In this research axis, it is part of our study whose goal is to valorize and improve the emulsifying properties of crude whey (acid and soft); that they underwent the microwave heating in order to select the best crude whey who have the high viscosities and to study the impact of the treatment on the size and distribution of the fat globules of the dispersed phase and the interfacial surface of continuous phase.

MATERIALS AND METHODS

Crude whey

Both types of whey are prepared in the laboratory from a powdered skim milk (0% fat) manufactured using cow's milk by FONTERRA Ltd, 9 Princes Street, Auckland, New Zealand [32-39]. The acid crude whey was prepared by adjusting the pH of the milk prepared 10% at the isoelectric pH of casein, for against the soft crude whey is made from the same type of milk by adding 2V of rennet (1%) , and it is heated to 35°C / 40min [33]; both types of whey were collected after filtering through filter paper (Folded Filters from Germany: 185mm diameter) and stored at 4°C [32-39]. The physicochemical tests applied to different types of whey are: The pH (pH meter 822 CG SGH), the lactose using the method as claimed [34], the proteins (method of [35]), the viscosity by viscometer of ball falling (viscometer: Hoesppler BH2), the ash content and dry matter by the method [36] and the density by the pycnometer [38].

Crude whey treated

In order to achieve the control of the viscosity kinetic (viscometer: Hoesppler BH2) and the temperature (thermometer KIKATRON) for both types of whey prepared; they are placed individually in glass vials (type Erlen Meyer DURAN SCHOTT MAINZ, JENA^{ER} GLAS) in the microwave oven (SAMSUNG, TDS: Triple Distribution System, Type-M1833N, JULY-2008), the operating conditions in the experiment are :2.45GHz and 800W, some physicochemical parameters are analyzed for crude whey (acid and soft) which recorded maximum viscosities namely: pH (pH meter CG 822 GHS), the lactose using the method according to [34], the proteins ([35], the ash content and dry matter by the method AFNOR [36] and the density by the pycnometer [38].

Virgin olive oil

For the dispersed phase of the emulsion, we chose a virgin olive oil produced locally in Algeria; extracted by cold pressed, its brand is "ZZIT UZEMMUR" produced by olive *Ifri* (Ighzej Amokrane, Bejaia, Algeria) by the standards of IOC (International Olive Oil Council), its physicochemical parameters are controlled at the laboratory to measure the pH (pH meter CG 822 GHS), the viscosity by a falling ball viscometer (viscometer: Hoesppler BH2), the density by pycnometer according to K. Karleskind [41] and the acidity index is determined according to the method described by AFNOR [37].

Emulsions

The emulsion is composed of a dispersed phase (virgin olive oil) and a dispersing phase (crude whey) treated and untreated by microwave heating; the dispersions are prepared in the presence or absence of sodium caseinate (stabilizing agent) following a ratio V / V equal to 0.0526%; each mixture was homogenized at 25 °C according to speed 8000 turn/30 min by a homogenizer (Ultra-Turrax T25, JANKE and KUNCKEL, IKALabortechnik). The average diameter of the fat globules is observed and determined under the light microscope (PHYWE, hund WETZLAR, GERMANY) using a micrometer graduated from 0 to 10, the graduations are spaced from each other of 0.1µm, and then the interfacial surface is calculated using the formula given by S. Arditty [17].

Statistical Analysis

The experimental data are analyzed statistically by R software.

RESULTS AND DISCUSSION**Crude whey treated**

Changes in the viscosity and temperature of the crude whey treated (acid and soft) are indicated by figure1. From figure 1, we note that the processing of crude whey (acid and soft) by microwave heating has caused the variability during the kinetic for controlled parameters (viscosity and temperature); maximum viscosity values were recorded as follows: 1.7996cP and 77 °C were labeled for crude acid whey treated during 45s, and 1.8611cP and 23°C were recorded for soft crude whey treated for 5s.

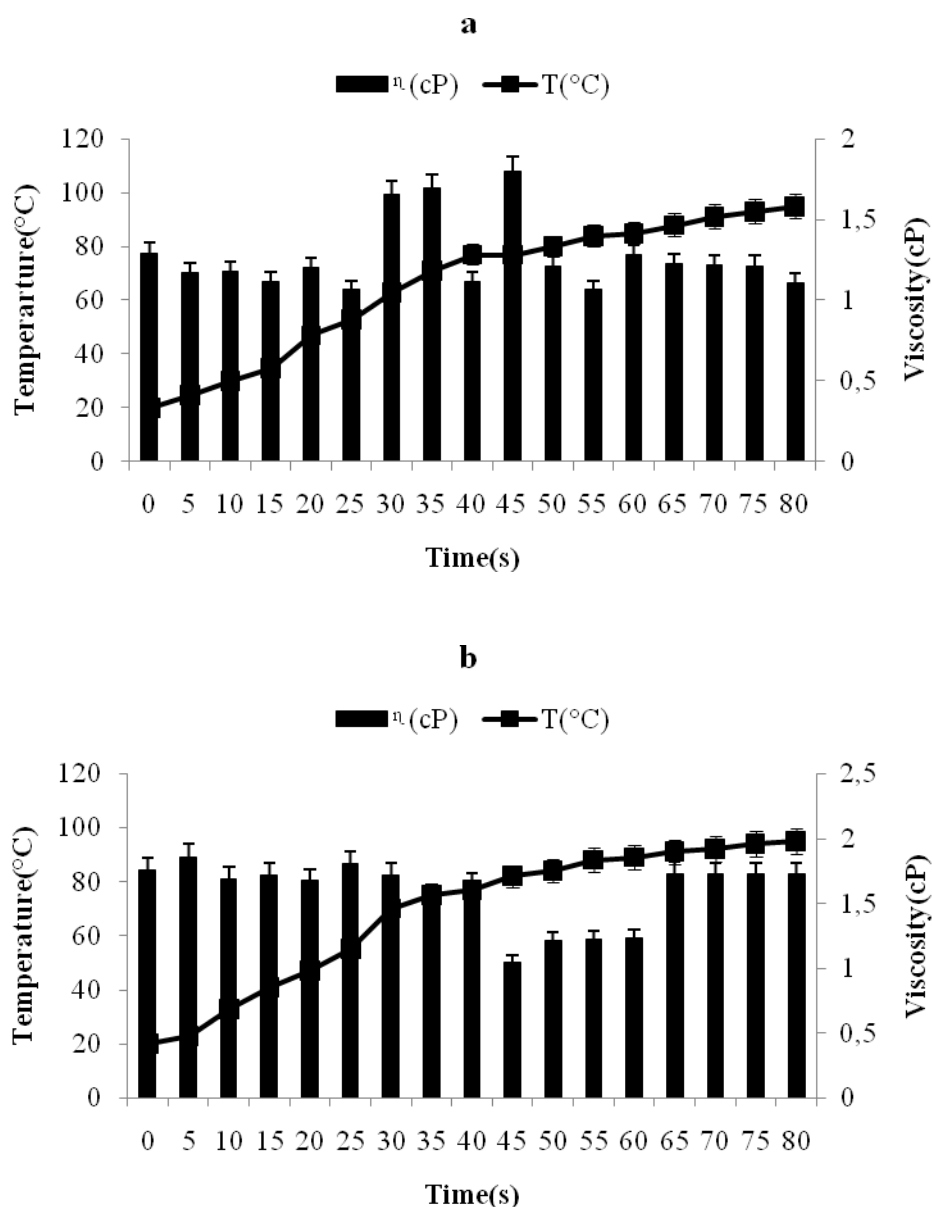


Figure 1: Kinetics of the viscosity and temperature of the crude whey treated by the microwave heating (a: Acid crude whey; b: Soft crude whey).

Some physicochemical parameters of crude whey treated (acid and soft) which showed maximum viscosity values are reported by figure 2.

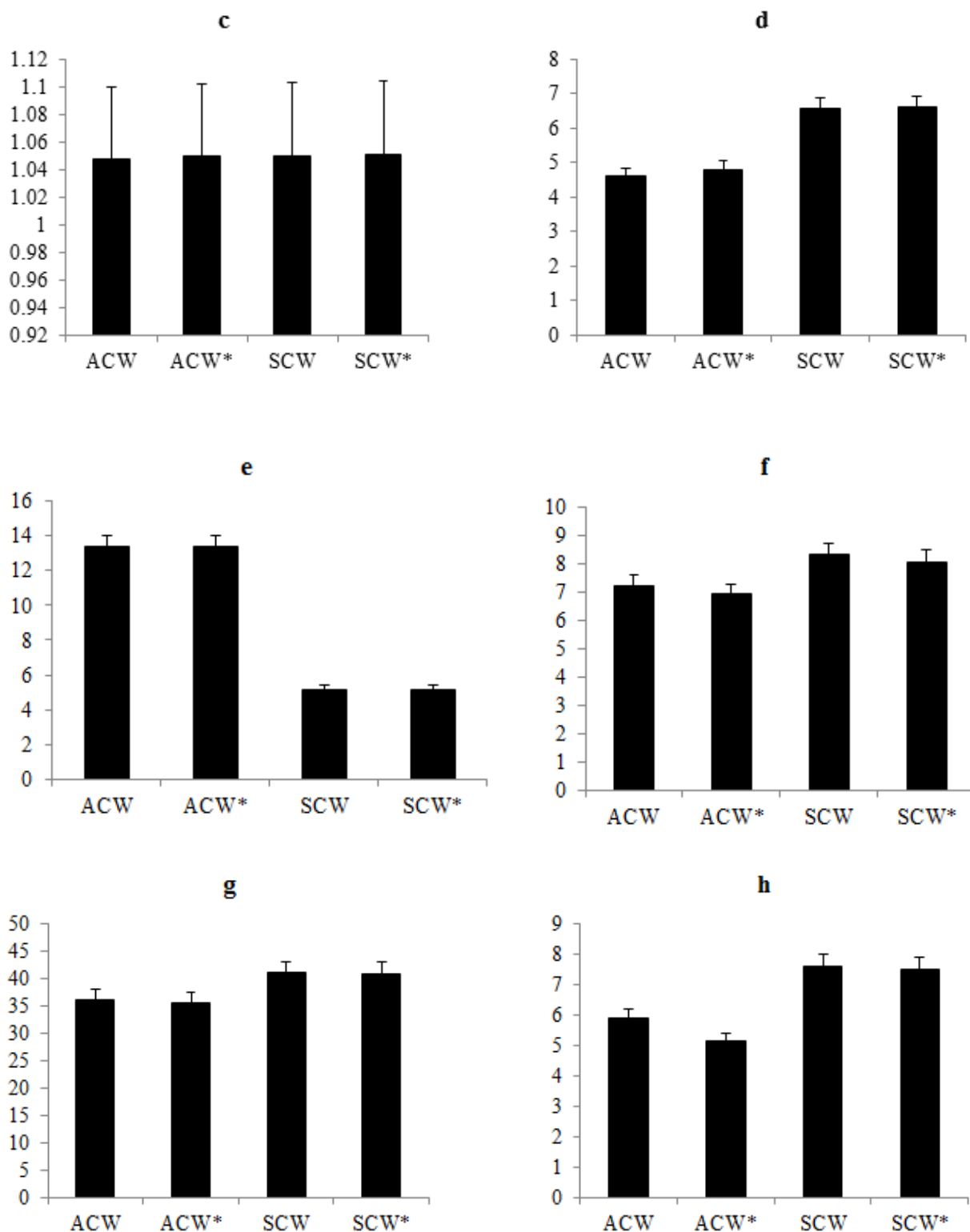


Figure 2: Behavior of some physicochemical parameters of crude whey treated by microwave heating (c: Density, d: pH, e: Ash (g/l) f: Proteins (g/l), g: Lactose (g/l), h: Dry matter (%))

The microwave heating of the crude whey (acid and soft) that have high viscosities have generated a slight increase in density (figure2c: 1.0468 to 1.0491 for the acid crude whey; 1.05 à 1.051 for soft crude whey) and pH (figure2d: 4.6 to 4.79 for the acid crude whey; 6.55 to 6.6 for the soft crude whey), stability of ash (figure2e: 13.35 to 13.35 g/l for acid crude whey; 5.12 to 5.12 g/l for soft crude whey) and decreased proteins (figure2f: 7.2 to 6.9 g/l for acid crude whey; 8.05 to 8.3 g/l for soft crude whey), lactose (figure2g: 36 to 35.5 g/l for acid crude whey; 41 to 40.75

g/l for soft crude whey) and the rate of dry matter (figure 2h: 5.9 to 5.12% for acid crude whey; 7.58 to 7.5% for soft crude whey). The results for the viscosity and temperature of the crude whey treated are consistent with those obtained by C. Bircanet *et al* [61], C. Zhou, [1] who found that the microwave heating induced a positive correlation between the water temperature and the time of treatment. According to J. Adrian [51], the viscosity depends on the temperature, the nature of the solvent, the size, shape, concentration, of the electric charge of the dispersed particles and their affinity for the solvent. According to D. Lorient [53], the viscosity of most proteins increases in an alkaline medium. According to J.F. Boudier [52], the density depends of the dry matter content, and fat content and the temperature. The increasing values of the density can make us conclude that the samples suffered a concentration process, mostly during the second minute of the experiment [47].

The pH values are comparable to those found by K. Acem [39]; pH is a decreasing function with the acidity, it evolves with the composition and the high content of acidic substances [38]; [54] underlined that the thermophilic ferments are less sensitive to pH than mesophilic: *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk develop respectively to pH 4.1 and 3.8; and according to A. Eck, [55], their ability to grow at higher temperatures than 40 °C, optimum growth is between 40 and 50 °C. Furthermore the ash content of the crude whey studied is comparable to those found by T. Croguennec [56] that have obtained the following results: 7.5g/l for soft whey, 12g/l of acid cheese whey and 9 g/l acid casein whey. According to [38], the heating of whey causes the destruction of organic matter: atoms of carbon, hydrogen and oxygen, lactose, fats, proteins, vitamins and other components are combined to form minerals; mineralization is the formation of body minerals from organic molecules. Values of the proteins belong to the interval given by D. Lorient [57] in the range of 0.9 to 13g/l; the results for the crude whey treated are comparable to those obtained by H. Kaddouri [49] which are characterized by a remarkable decrease that are linked with frequency, power and exposure time. According to [58], whey proteins denature more easily due to the temperature. If the pH of the medium is less than the pHi soluble proteins whey; they are found ionized form where the cationic form is predominant [59]. Moreover, the proteins macromolecules generally have good interfacial properties because they are in fact composed of hydrophobic areas (presence of proline, leucine, isoleucine, tryptophan, phenylalanine) and hydrophilic areas (the presence of aspartic acid, glutamic acid, phosphoserine), hydrophilic areas of globular proteins are exposed to the aqueous solvent, whereas the hydrophobic areas are most often located in the heart of the structure having minimum contact with water. Furthermore, the results obtained by D. Pomerai [48] shows that microwave increase the denaturation rate of whey proteins such bovine serum albumin.

The decreasing of the proteins concentration can be explained by the fact that the whey proteins sulfhydryl groups, typically buried within the core of the proteins structure, are exposed to the surface because of heating, furthermore, proteins sulfhydryl groups may be formed because of hydrolysis or β -elimination of disulfide bonds during thermal treatment; β -lactoglobulin, a major protein component of the whey fraction is often involved in these processes [47]. [50] suggested that microwave treatment could non thermally affect the kinetics of the β -lactoglobulin folding process, also, some of the hypotheses behind non thermal effects of microwave heating suggest that microwave radiation may change the solvent properties of water. The lactose content in the crude whey is lower than those found by [56] that have found the following values: 50g/l for soft whey and 44g/l for acid whey of cheese, and 50g/l is recorded for acid whey of caseins. The explanation for the decreasing lactose concentration under the microwave treatment of the milk samples could be the fact that lactose, being a reducing disaccharide, can be involved in the Maillard reaction and during this reaction's first stage lactose is isomerised in lactulose [47]. The results of the content of dry matter are comparable to those found, [47] who found that heating the milk to microwave (2.45GHz, 800W) decreases over time the rate of dry matter; the decreasing values of the dry substance are a confirmation of the fact that the samples lost quantitatively: water, proteins and lactose from their composition, processes demonstrated before, also, the decreasing values of the dry substance can be influenced by the modifications that can appear concerning the vitamin content of the samples. According to [60], the change in the concentration of whey dry matter essentially depends on the quality of milk used and the method of separation of whey.

Virgin olive oil

The figure 3 gives some physicochemical properties of virgin olive oil.

The average value of the virgin olive oil viscosity (71.92cP); it is less than the interval given by [45] ranges from 75-79 cP and it is thus higher than that published [41] that is limited 51-62cP; [41] the viscosity depends on the chemical structure of the fatty substance, the temperature, it increases with the molecular weight and decreases with increasing the number of unsaturation, the acidity index of virgin olive oil is equal to 0.84%; this value is consistent with the standard cited [43-44], which require a value lower than 2%; in general, the acidity index of an oil depends on its chemical composition and its storage conditions [41].

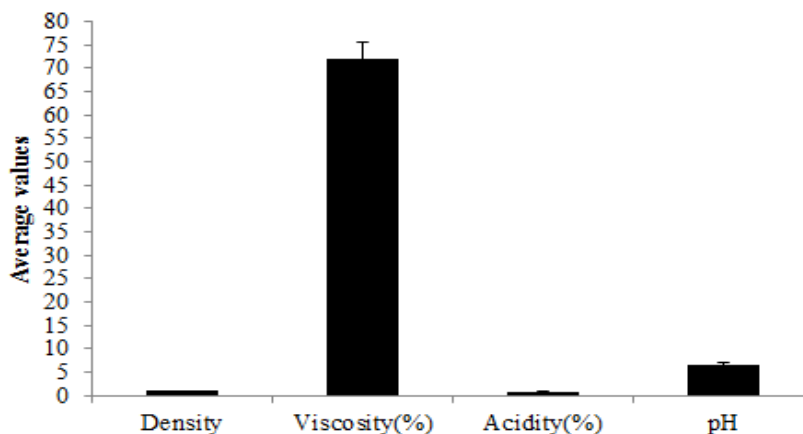


Figure 3: Average values of some physicochemical parameters of virgin olive oil measured at 20°C

The density (0.91) obtained is comparable to the range found by [45] that ranges from 0.910-0.916; according to [41], the density of a fat depends on its temperature and chemical composition, and it is lower compared [46], who noted that the density of the vegetable oil ranges from 0.915 to 0.964. We note that the pH of virgin olive oil is similar to that of neutrality, which is better suited to the skin [42].

Emulsions

Figures 4 and 5 respectively show the kinetics of interfacial surface and diameter of the fat globule of emulsions. We find that the curves of the interfacial surface are decreasing (figure 4) contrary to those of the fat globule diameter (figure 5) which are characterized by an increase over time.

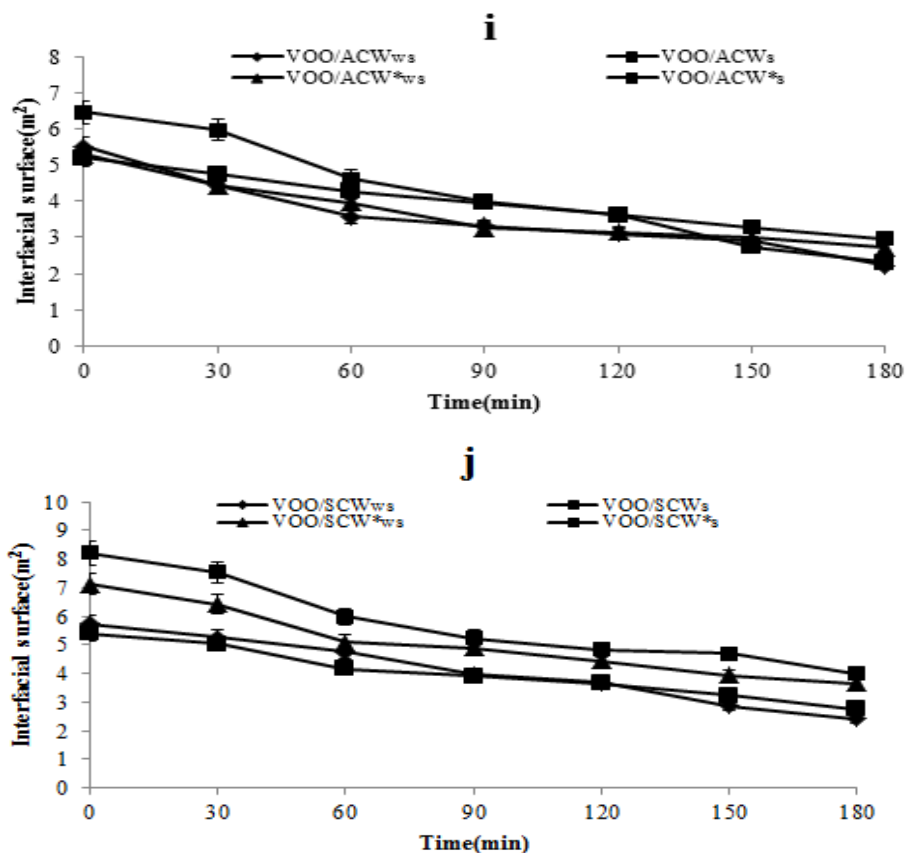


Figure 4: Kinetics of interfacial surface of emulsions (i: Acid crude whey, j: Soft crude whey)

Such high viscosity inhibits the interfacial rearrangements and generally the viscosity of surface mixture is always lower than the sum of the viscosities of the components [69]; this would explain our observations on the work emulsions. The results of [62] showed that the egg white proteins hydrolyzed and treated by microwave compared to those treated with conventional heating showed a high solubility and excellent emulsifying properties. The droplet size is another factor that may influence the viscosity; some authors [64-65] have shown that the viscosity is higher for emulsions small drops than to large drops. The rheological behavior of an emulsion often depended on the viscosity of the external phase [63]. [13] have shown that negative correlation is shown between the viscosity of the external phase of the emulsion and the size of globules fat dispersed.

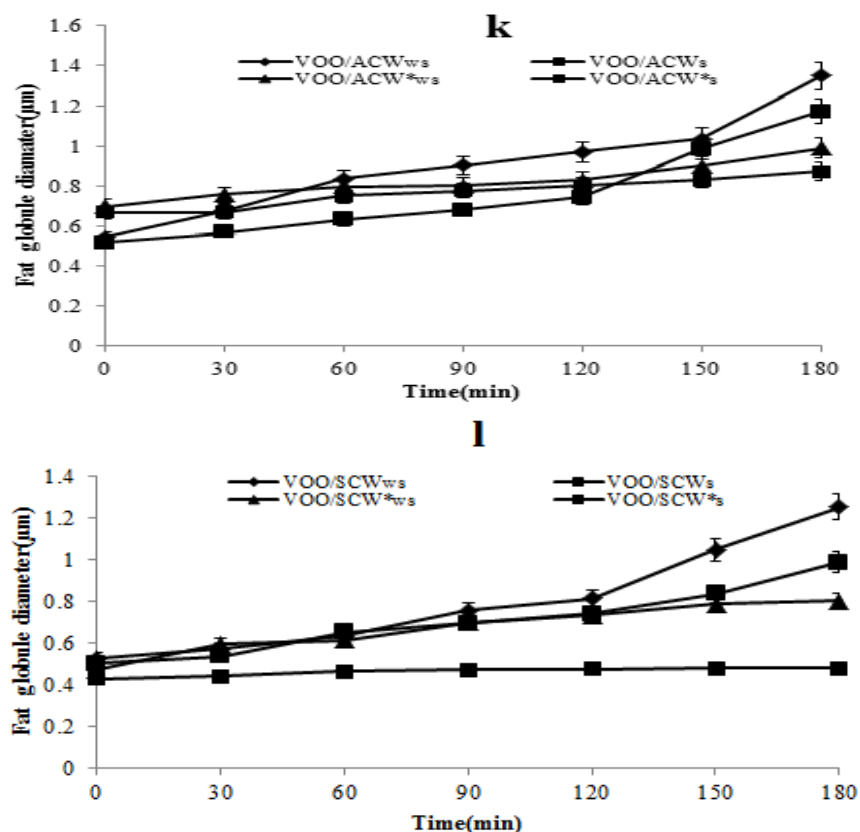


Figure 5: Kinetics of the diameter of the fat globule of emulsions (k: Acid crude whey, l: Soft crude whey)

The table 1 shows the Anova test for the interfacial surface which we have defined three same highly significant correlation ($< 2.2 \times 10^{-16}$ ***): emulsion at base of acid crude whey untreated by microwave with stabilizer, emulsion at base of acid crude whey treated by microwave at 45s without and with stabilizer, but the emulsion at base of soft crude whey untreated by microwave with stabilizer has noted a significant correlation (0.02756^*), in opposite there is two emulsions prepared at base of soft crude whey without and with stabilizer have marked two same significant correlation ($< 2.2 \times 10^{-16}$ ***) for the diameter of fat globule (table 2).

Table 1: Anova test for the interfacial surface of emulsions at base of crude whey witness and treated by microwave

Samples	Df	SumSq	MeanSq	F value	Pr (>F)
VOO/ACW					
VOO/ACWs	1	6.2976	6.2976	$1.3088e^{+32}$	$< 2.2e^{-16}$ ***
VOO/ACW*ws	1	2.8324	2.8324	$5.8864e^{+31}$	$< 2.2e^{-16}$ ***
VOO/ACW*s	1	0.5843	0.5843	$1.2143e^{+31}$	$< 2.2e^{-16}$ ***
Residuals	3	0.0000	0.0000		
VOO/SCW					
VOO/ SCWs	1	9.0000	9.0000	16.2000	0.02756 *
VOO/ SCW*ws	1	0.1429	0.1429	0.2571	0.64702
VOO/ SCW*s	1	0.0476	0.0476	0.0857	0.78878
Residuals	3	1.6667	0.5556		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table2: Anova test for the fat globule diameter of emulsions at base of crude whey witness and treated by microwave

Samples	Df	SumSq	MeanSq	F value	Pr (>F)
VOO/ACW Residuals	6	8.0749e ⁻³²	1.3458e ⁻³²		
VOO/SCW VOO/SCW*ws	1	8.0749e ⁻³²	8.0749e ⁻³²	2.4798e ⁺⁶⁵	< 2.2e ⁻¹⁶ ***
VOO/SCW*s	1	0.0000e ⁺⁰⁰	0.0000e ⁺⁰⁰	3.2198e ⁺³³	< 2.2e ⁻¹⁶ ***
Residuals	4	0.0000e ⁺⁰⁰	0.0000e ⁺⁰⁰		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

The tables 3 and 4 respectively show the statistical description of the interfacial surface and the diameter of fats globules of emulsions at base of crude whey witness and treated by microwave.

Table3: Statistical description of the interfacial surface of emulsions at base of crude whey witness and treated by microwave

Variables	Average	Minimum	Maximum	Standard Deviation
VOO/ACWws	3.576286	2.220000	5.504000	1.083495
VOO/ACWs	3.834571	2.303000	5.205000	1.040133
VOO/ACW*ws	3.680000	2.710000	5.290000	0.921267
VOO/ACW*s	7.414286	2.960000	6.450000	1.337670
VOO/SCWws	4.096000	2.399000	5.736000	1.226748
VOO/SCWs	4.014571	2.743000	5.389000	0.941695
VOO/SCW*ws	5.080000	3.650000	7.130000	1.277928
VOO/SCW*s	5.778571	3.980000	8.210000	1.562663

Table4: Statistical description of the fat globule diameter of emulsions at base of crude whey witness and treated by microwave

Variables	Average	Minimum	Maximum	Standard Deviation
VOO/ACWws	0.903143	0.545000	1.351000	0.260223
VOO/ACWs	0.757714	0.518000	1.172000	0.237451
VOO/ACW*ws	0.824000	0.697800	0.990400	0.095974
VOO/ACW*s	0.767071	0.669000	0.872800	0.076883
VOO/SCWws	0.798429	0.523000	1.250000	0.265420
VOO/SCWs	0.705429	0.501000	0.984000	0.167813
VOO/SCW*ws	0.669871	0.470200	0.800200	0.118037
VOO/SCW*s	0.462171	0.428300	0.479900	0.019921

By thermal denaturing treatment, it is thus possible to increase the water retention and the surfactant properties, provided it is carried in pH out at different of pH_i (5.2 for the β -lactoglobulin, 4.8 for α -lactalbumin), the β -lactoglobulin is usually best as a surfactant that α -lactalbumin, especially if it is purified; moreover, the mobility of the α -lactalbumin is greater than that of β -lactoglobulin and that the effects are most pronounced near the pH_i, due to the decrease of the electrostatic repulsion [68]. [66] note that the unfolding of β -lactoglobulin and α -lactalbumin by reduction and carboxymethylation SS bridges also increases the flexibility of molecules, whereas heat denaturation does not appear to have significant effects unless the proteins precipitate, in which case the mobility is greatly reduced, these observations on the mobility and flexibility can be easily connected to the structure not only more or less of unfolded molecules, but also is linked at interfacial behavior which the surfactant properties are the best when the mobility is higher (flexible proteins such as casein and unfolded globular proteins, pH_i, presence of salts, etc). [67] have shown that flexibility appreciates the dynamic state of the protein and its ability to interact with other molecules of the same or of a different nature (water, salts, carbohydrates, lipids, surfactants). [66] conclude that the emulsifying activity and foaming ability of the purified proteins were higher than the witness whey, at the same of protein concentration (minimum at pH_i for emulsifying activity, maximum at pH_i for foaming capacity), it is the same for the emulsifying and the foaming stability [68]; in mixture, it is found that the β -lactoglobulin is mainly adsorbed on the fat globule unless at acidic pH, irrespective of the concentration ratios of the two proteins [70]. These proteins form elastic and viscous interfacial films (1100 and 350 mN.s.m⁻¹ for the β -lactoglobulin and the α -lactalbumin, respectively) against 100 mN.s.m⁻¹ for gelatin, 8 mN.s.m⁻¹ for caseinate and 0.5 mN.s.m⁻¹ for β casein [66]. The microscopic appearance of emulsions at base the crude whey as witness and treated is illustrated by figure 6.

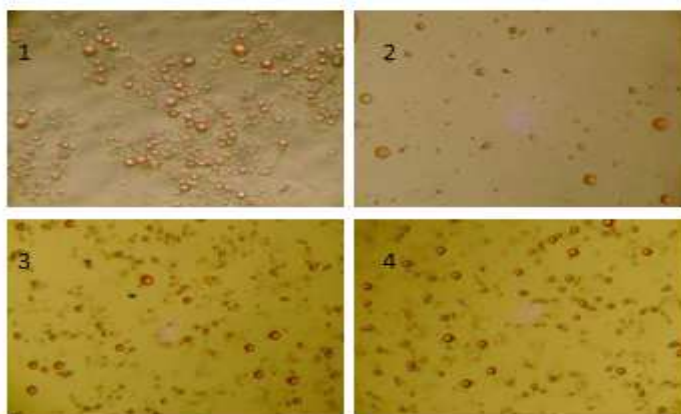


Figure6:Microscopic aspects(Mgx10)ofemulsionsVOO/CW showing the influence of microwave heating on the viscosity of the continuous phase and her emulsifying properties over time:

1:EmulsionVOO /ACWs($\eta_{cp}=1.292cP$,proteins=7.2g/l, $\Phi=0.757714\pm0.237451\mu m$, $IS=3.834571\pm1.040133m^2$) ;2 :EmulsionVOO /ACW*s($\eta_{cp}=1.7996cP$,proteins=6.9g/l, $\Phi=0.767071\pm0.076883\mu m$, $IS=7.414286\pm1.337670m^2$) ;3 :EmulsionVOO /SCWs($\eta_{cp}=1.7602cP$,proteins=8.3g/l, $\Phi=0.705429\pm0.167813\mu m$, $IS=4.014571\pm0.941695m^2$) ;4 :EmulsionVOO /SCW*s($\eta_{cp}=1.8611cP$,proteins=8.05g/l, $\Phi=0.462171\pm0.019921\mu m$, $IS=5.778571\pm1.562663m^2$) .

CONCLUSION

Analysis of the composition of crude whey (acid and soft) has freed a variability which depends essentially on their processes for obtaining and their origin, in addition the physicochemical properties of virgin olive oil controlled are compliant with norms. The present study showed that microwave heating has increased the viscosity of crude whey (acid and soft) compared to that measured for the crude whey (acid and soft) untreated; this treatment showed its effect on some physicochemical properties of crude whey (acid and soft) which is characterized by a slight decrease in proteins, lactose and dry matter, stability of the ash content and a slight increase in the pH and density, the combination of crude whey (acid and soft) treated by microwave and the operating conditions implemented have generated an improved their emulsifying behavior.

List of abbreviations

ACW*: Acid crude whey treated by MW during 45s

ACW: Acid crude whey as witness

CW: Crude whey

IS: Interfacial surface

Mg: Magnification

MW: Microwave

SCW*: Soft crude whey treated by MW during 5s

SCW: Soft crude whey as witness

VOO /ACW*s: Emulsion of virgin olive oil in acid crude whey treated by MW during 45s with stabilizer

VOO /ACW*ws: Emulsion of virgin olive oil in acid crude whey treated by MW during 45s without stabilizer

VOO /ACWs: Emulsion of virgin olive oil in acid crude whey as witness with stabilizer

VOO /ACWws: Emulsion of virgin olive oil in acid crude whey as witness without stabilizer

VOO /SCW*s: Emulsion of virgin olive oil in soft crude whey treated by MW during 5s with stabilizer

VOO /SCW*ws: Emulsion of virgin olive oil in soft crude whey treated by MW during 5s without stabilizer

VOO /SCWs: Emulsion of virgin olive oil in soft crude whey as witness with stabilizer

VOO /SCWws: Emulsion of virgin olive oil in soft crude whey as witness without stabilizer

VOO/CW: Emulsion of virgin olive oil in crude whey

VOO: Virgin olive oil

η_{cp} : Viscosity of the continuous phase

Φ : Diameter of the fat globule

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