Study of the effect of power’s ultrasound on the protein's emulsifying properties of crudes wheys

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ABSTRACT

The study of the effect of power’s ultrasound set to 35KHz on the protein’s emulsifying properties of crudes wheys (acid and soft), allows us to achieve two main parts, the first part is the characterization of the phases of the emulsion are sweet almond oil and crudes wheys (acid and soft), and the second part focused on the study of the emulsifying power of crudes wheys proteins (acid and soft) untreated and treated by ultrasound. The results show that the physicochemical parameters of sweet almond oil are suitable for emulsification, the values of the physicochemical parameters of the crude soft whey are higher than those found in whey including crude acid whey: proteins, lactose, density and viscosity. We record the values of the interfacial tension slightly stable in crudes wheys. No change was noted for the pH values of the crudes wheys sonicated during 5, 10 and 15min, except that their temperature undergoes a slight increase. Emulsion stability of O / W type prepared with crudes wheys treated and untreated by ultrasound varies with physicochemical environment, biochemical wheys used and the presence or absence of sodium caseinate as a stabilizer, or the ultrasound treatment of crudes wheys (acid and soft) has improved the average stability of the emulsions according to the exposure time of 5,10 and 15min.

Key words: crudes wheys, sweet almond oil, power’s ultrasound, environment, emulsion, stability, proteins.

Introduction

The cheese whey is an industrial waste by its fermentable organic matter is the positive factor of biological pollution of freshwater ecosystems. The amount of whey in Algeria as in other countries of the world accounts for approximately 85% of the milk into cheese, whey is however an interesting product for its protein rich in essential amino acids (lysine and tryptophan), lactose, minerals and the presence of numerous B vitamins such as thiamine and riboflavin [21].

Emulsions are inherently unstable systems that can destabilize next several mechanisms reversible or not; among these mechanisms, the main two are creaming (or sedimentation following the relative densities of the two liquids) and coalescence, creaming is the migration droplets upwards under the effect of gravity which is due to the different densities of the two phases, dispersed and continuous sedimentation phenomenon is the same but in this case, the droplets migrate downwards; coalescence is the fusion of two droplets to form a larger one, different destabilization phenomena result in changes to both the size and number of droplets in the emulsion and by changes in the microscopic appearance of emulsions. The study of the stability of emulsions is therefore essential to understand the parameters to get systems that meet the stability criteria defined a priori, various methods have been proposed to monitor the emulsion destabilizing including conductivity measurements of turbidity or ultrasonic techniques [43].

Among the techniques that enable activation process intensification physico-chemical, power ultrasound are known to increase the conversion and / or selectivity of many chemical reactions and to improve various physical processes due to cavitation effects; the power’s ultrasound , high intensity and low frequency from 16 at 1000KHz and in this frequency range, the desired effect is a modification of the environment by ultrasound cavitation mainly due to: the wave modifies the irradiated environment, this change may physical (pickling, degassing, emulsification) or chemical (change of the reaction mechanism, production of free radicals ...) is the field of use of ultrasound in process engineering, the best known example is the most widely used and

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ultrasonic cleaning operating at frequencies below 50 kHz, however, it is not uncommon practice sonochemistry at frequencies up to and mega hertz these high frequencies seem more favorable to the production of free radicals, and will preferred methods for example by degradation of chemical pollutants [46].

Moreover, the study of ultrasonic inactivation of bacterial species most active in milk as Pseudomonas fluorescens and Streptococcus thermophilus, was conducted by Villamiel and De jong [53] and Ferrante et al. [22] able to deduce that the ultrasonic treatment combined with natural antimicrobial (vanillin and / or citral) inactivates Listeria monocytogenes and is an alternative to conserve juice. The same polymers are devolatilized (evaporation of monomers) and water can be deaerated, usually to prevent corrosion or adverse reactions due to dissolved oxygen, however most of these applications are not yet developed stage industrial. The degassing effect of ultrasound is very promising, yet there is no clear physical representation of the ultrasonic degassing mechanism or link between the parameters of the ultrasonic field and their implications for degassing, and in a manner general literature on this subject is scarce [28,48,24]. Several studies are carried out on the functional behavior and individual caseinates and whey protein concentrates in aqueous solution and many journals have already established inventory hydration properties, and surface texture [13,23,30,17,30,39,6,15,19,32,2,7,55]. Additional changes induced by ultrasound in the field of chemistry are studied in other fields, few data are available on their effects on food constituents [45], the recent work of Rezek Jambrak et al. [49] showed the effect of ultrasound on improving the solubility and foaming properties of whey proteins. In this context it fits our study's main objective is to improve the emulsifying properties of whey proteins whose research focuses on the characterization of crude wheys (acid and soft) and control stability of emulsions prepared with crude wheys (acid and soft) untreated and treated by ultrasound.

Materials and Methods

Crudes wheys:

The two types of whey are prepared in the laboratory from a powder skim milk (0% fat) manufactured using cow's milk by FONTERRA Ltd, 9 Princes Street, Auckland, New Zealand [1,2]. The crude acid whey (LSAB) is prepared by adjusting the pH of the reconstituted milk 10% at the isoelectric pH of the insoluble proteins against crude soft whey (LSDB) is prepared from milk of the same type by adding rennet 2Vde 1% and heated to 35 °C/40min (Baumy and Brule, 1986), and these types of whey were recovered after filtration by simple filter paper (Folded filters from Germany: 185mm diameter) and they are stored at 4°C [1,2]. The physico-chemical tests and biochemical applied to different types of whey and sweet almond oil are: pH (pH meter CG 822 GHS), lactose using the DNS method according to Miller [38], proteins [36], the Calcium content (flame photometer Jenway PEP 7), the viscosity by a falling ball viscometer (viscometer: Hoeppler BH2), the ash content by the method Afnor [4], the interfacial tension is determined by bidimensional Dunouy 70545, density by pycnometer according to Hardy [25] and the acidity value is determined using the method described by Schnadeltach [52].

Emulsions:

The emulsion is composed of a dispersed phase (oil sweet almond provided by the Algerian trade; Mazouna origin, Algeria) and a dispersing phase (crudes wheys) untreated and sonicated (unit: TH52 SONOREX fixed 35KHz) during 5,10 and 15 min; the dispersions are prepared in the presence or absence of sodium caseinate (stabilizer) at a ratio V / V equal to 0.0526%, and each mixture was homogenized at 25 °C according to the speed 8000tours / 30min by a homogenizer (Ultraturrax JANKE and Kunckel, IKA, labotechnik). Emulsion stability is a spectral measure determined using a spectrophotometer BAUSCH and LOMB , spectronic 70 and is calculated following the formula given by Pearce and Kinsella [44] whose data are statistically analyzed by software R (test ANOVA).The average diameter of the fat globules is determined using an ocular micrometer calibrated from 0 to 10 whose graduations are spaced from each other to 0.1μm [2].

Results and Discussion

Crudes wheys:

Table 1 shows the physico-chemical and biochemical means of whey studied.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>pH at 25°C</th>
<th>Calcium (g/l)</th>
<th>Ashes (g/l)</th>
<th>Proteins (g/l)</th>
<th>Lactose (g/l)</th>
<th>Viscosity (cP) at 25°C</th>
<th>Density at 25°C</th>
<th>Interfacial tension (Dynes/cm) at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSAB</td>
<td>4.65</td>
<td>0.0255</td>
<td>7.97</td>
<td>7</td>
<td>47.36</td>
<td>1.223</td>
<td>1.027</td>
<td>35.5</td>
</tr>
<tr>
<td>LSDB</td>
<td>6.72</td>
<td>0.086</td>
<td>6.82</td>
<td>13</td>
<td>48.42</td>
<td>1.323</td>
<td>1.081</td>
<td>34.4</td>
</tr>
</tbody>
</table>
**pH and lactose:**

Physico-chemical and biochemical crudes wheys (acid and soft) study are comparable to those found by Acem and Choukri [2] and they are characterized by a remarkable variability, pH = 4.65 for LSAB against pH = 6.72 for LSD; the gold levels of lactose in crudes wheys are lower than those found by Crouguennec et al. [16] found that the following values: a value of lactose 50 g / l for soft whey and acid whey cheese, its content equal to 44g / l whose 50 g / l of lactose is recorded for acid whey from casein. The pH is a decreasing function with acidity, it evolves with composition and high content of acidic substances [37], according to Multon [41], the thermophilic ferment are less sensitive to pH than mesophilic: *Streptococcus thermophilus and Lactobacillus bulgaricus* grow in milk respectively to pH 4.1 and 3.8, and by Eck and Gillis [20], their ability to grow at temperatures above 40°C, the optimal growth between 40 and 50°C. The results of the physicochemical analysis of soft whey from the manufacture of the pie from the Draa Ben Khedda ORLAC of Tizi Ouzou in Algeria determined by Boudjema et al., [11] show that this medium culture has decent quality saw its high nutritional contents: 57.9 g / l of lactose, 1.12 g / l of nitrogen, 7 g / l of proteins, 1.75g / l of chloride and 0 g / l of fat.

**Ashes and calcium:**

In addition, the ash content of whey studied are lower than those found by Crouguennec et al., [16] obtained the following results: 7.5g / l for soft whey, 12g / l for acid whey cheese and 9g / l for the acid whey from casein. The calcium found values for all whey are lower compared to those obtained by Britten [12] which are values of 0.05% and 0.1% calcium respectively for soft whey and acid whey, and the Fao [21] notes that the calcium content of whey varies from 0.5 to 0.1g / l; Saulnier et al., [51] show that the calcium content of whey (acid and soft) varies with treatment changes (concentration); Pouliot et al., [47] note that a pH range between 6.6 and 8.0 at 25 and 50°C, it is clear that the conditions pH 8.0 and 50°C precipitation caused the most complete, 61% calcium and 32% of soluble phosphate.

**Viscosity and density:**

The values of the viscosity and density for crudes wheys are respectively: 1.223cP and 1.027 for LSAB, 1.323cP and 1.031 for LSD, according Adrian et al., [3], the viscosity depends with 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 Lorient et al., [34], the viscosity of most proteins increases in urban alcalin. According to Boudier and Luquet [10], the density depends on the dry matter content, and fat as well as temperature.

**Proteins and interfacial tension:**

The proteins content of the LSAB is lesser than that found in LSDB: 7.12g / l for LSAB against 13g/l for LSDB, if the pH of the medium is lesser than the pH of soluble whey proteins, they are ionized form which cationic form predominates and according Jeantet et al., [27], whey proteins negatively charged at pH = 6.5 (β-lacoglobuline, α-lactalbumin) are retained on an anion exchange resin by lactose and cons whey proteins positively charged at pH = 6.5 (10% whey proteins) through the resin without retenus. For surface properties: sweet almond oil / whey, all interfacial tensions are lower than that found obtained for sweet almond oil / water is 45 Dynes / cm whose the values of the interfacial tension varies from one whey to another: 35.5 Dynes / cm for LSAB and 34.4 Dynes / cm for LSDB, according Jeantet et al., [26] and Jeantet et al., [27], at the interface between two phases (liquid / gas, liquid / liquid immiscible), the molecules are in an asymmetrical and the attractive forces exerted by each of the phases of the molecules located at the interface are different, resulting in an energy or interfacial tension which corresponds to an energy per unit area, on the other hand the organic molecules are preferentially concentrated at the interface and lower the interfacial tension in the case they have hydrophilic and hydrophobic areas (amphiphilic character). However Jeantet et al., [27] point out that the interfacial tension decreases with the concentration of the solute in solution until a level corresponding to the organization of amphiphilic molecules into micelles, which limit concentration is called concentration critical micelle (CMC), in addition to the phenomena of diffusion and adsorption of molecules at the interface mechanisms are often slow and must move solvent molecules initially present and subject, in the case of macromolecules conformational changes that can facilitate the formation of intermolecular interactions up to the formation of a cohesive interfacial film, also the proteins macromolecules generally have good interfacial properties because they are in fact composed of hydrophobic regions (presence of proline, leucine, isoleucine, tryptophan, phenylalanine) and hydrophilic areas (presence of aspartic acid, glutamic acid, phosphoserine), the hydrophilic regions of globular proteins are exposed to the aqueous solvent, while the hydrophobic regions are most often located in the heart of the structure with the minimum contact with water.

**Sweet almond oil:**
Table 2 shows some physicochemical parameters means the commercial almond oil studied.

Table 2: Physicochemical parameters means the commercial almond oil studied.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sweet almond oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (cP)</td>
<td>69.0563</td>
</tr>
<tr>
<td>Acidity (%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Density</td>
<td>0.9153</td>
</tr>
<tr>
<td>pH</td>
<td>3.83</td>
</tr>
</tbody>
</table>

The average value of the viscosity of sweet almond oil is slightly lower than that found by Morin [40], who found a value 71cP, according Karleskind [29], the viscosity varies from 68 to 76 cP at 20°C and it is dependent upon the chemical structure of the body fat, the temperature and molecular weight resulting in an increase in viscosity. The acid of sweet almond oil is equal to 0.72%, and this value is consistent with the standard cited by Afnor [5] that requires a value lower than 2%, in general, the index acidity of an oil depends on its chemical composition and storage conditions [29]. The density obtained is comparable to that found by Morin [40], who found a value of 0.911-0.917 for sweet almond oil, according to Karleskind [29], the density of fat depends on its temperature and its chemical composition, and according Roger [50], the density of the vegetable oil ranges from 0.915 to 0.964. We note that the pH of the sweet almond oil studied was similar to that of neutrality, which is better suited to the skin [52].

Stability of emulsions:

We note that all the curves of the stability of emulsions prepared with the crudes wheys (acid and soft) and crudes wheys (acid and soft) treated with ultrasound is decreasing over time (figure1).

![Fig. 1](stability_emulsions.png)

Fig. 1: Stability of emulsions of crudes wheys untreated (A) and treated (A1, A2 and A3) by ultrasound in the presence (as) or absence (ss) of sodium caseinate.

The stabilities emulsions prepared with the crudes wheys (LSAas and LSDas) untreated by ultrasonic are characterized by variables decreased after 2 hours, stability and a decrease after 6 hours (Table 3; LSAas which case $MS = 75.6\%$ ) against the LSDas ($MS = 72.8\%$ ) was characterized by a decrease after 2 hours and high stability to 8 hours. The ultrasonic treatment after 5min of crude whey improved emulsifying behavior of whey proteins in emulsions studied especially: a stability constant for almost LSDas ($MS = 91.6\%$ ) and LSDas ($MS = 89.6\%$ ) and another in decreasing over time for LSAas ($MS = 64.2\%$ ). The crudes wheys sonicated after 10 minutes have led to a marked improvement in the stability of the emulsion made containing the LSDas ($MS = 95.2\%$ ) over time by cons decreased stability after 2 hours for it to be stabilized after 6 hours (LSDas which case $MS = 72.4\%$ ). Yet ultrasonic treatment of crudes wheys after 15min improved especially: values the stability of the emulsion prepared LSAas basis ($MS = 90\%$ ) is maintained until 4 hours then lowers and it becomes stable when 6 hours but the stability of the emulsion made based LSAss ($MS = 76\%$ ) decreases from its preparation and it retains its appearance from 2 hours to 8 hours, in addition to
that prepared from LSDss \( (MS = 77.4\%) \) was characterized by a decreasing pace after 2 hours.

**Table 3:** Descriptive statistics of stability (%) of emulsions prepared with crudes wheys untreated and treated by ultrasound.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Average</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSDss</td>
<td>66.0 62.1 76.3</td>
<td>38.6 52.5 70.8</td>
<td>100.1 100.1 100.1</td>
<td>27.5 17.5 21.5</td>
</tr>
<tr>
<td>LSDs</td>
<td>78.6 61.6 80.4</td>
<td>50.5 50.5 60.5</td>
<td>100.1 100.1 100.1</td>
<td>24.1 21.1 10.1</td>
</tr>
<tr>
<td>LSDss</td>
<td>72.2 60.2 77.4</td>
<td>32.6 92.5 78.5</td>
<td>100.1 100.1 100.1</td>
<td>25.5 6.0 7.0</td>
</tr>
</tbody>
</table>

The table 4 shows the ANOVA test for different types of wheys treated and untreated by ultrasound during 5, 10, and 15 min; the best results is defined respectively by the crudes wheys untreated by ultrasound: the crude acid whey stabilized by the sodium caseinate is highly significant \( (0.008933**) \) and the crude soft whey no stabilized by the sodium caseinate is significant \( (0.048532*) \) compared to the other wheys.

**Table 4:** Anova test of crudes wheys untreated and treated by ultrasound during 5, 10 and 15 min

We can say that the ultrasonic treatment is based on a large number of methods for improving functional properties (emulsifying); the sonication is used to suppress the resistance of milk serum protein proteolysis [42,33]. By thermal denaturing treatment, we can increase the power and water retention, and surfactant properties, provided it is carried out at different pH values of pHi (5.2 for \( \beta \)-lactoglobulin, 4.8 for \( \alpha \)-lactalbumin), the \( \beta \)-lactoglobulin is usually better as a surfactant that \( \alpha \)-lactalbumin, especially if it is purified; Moreover, the mobility of the \( \alpha \)-lactalbumin is higher than that of \( \beta \)-lactoglobulin and effects are most pronounced near the pHi, due to the decrease in electrostatic repulsion [14]. Lorient et al., [34] note that unfolding of the \( \beta \)-Lactoglobulin and \( \alpha \)-lactalbumin by reduction and carboxymethylation S-S bridges also increases the flexibility of the molecules, while thermal denaturation does not seem to significant effects unless the proteins precipitate which case the mobility is greatly reduced; these observations on mobility and flexibility can be easily connected not only to the structure more or less deshinter molecules, but also because the interfacial behavior surfactant properties are best when mobility is higher (flexible proteins such as casein and globular proteins deshired, pH, presence of salts, etc.). Blond and Le Meste [9] have shown that flexibility so appreciate 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). Lorient et al., [34] conclude that the emulsifying activity and foaming ability of the purified proteins are superior to whey witness to the same protein concentration (minimum pHi for emulsifying activity, maximum capacity for pHi foam), it is the same for emulsifying and foaming stabilities [14], in a mixture, we find that the \( \beta \)-lactoglobulin is mainly adsorbed on the fat globules, except at acidic pH, regardless of the concentration ratios of two proteins [32]; these proteins form elastic and viscous interfacial films (1100 and 350 mN.s.m\(^{-1}\) for \( \beta \)-lactoglobulin and \( \alpha \)-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 \( \beta \)-casein [34]. As high viscosity hinders interfacial rearrangements, in general, the surface
viscosity of the mixture is always lower than the sum of the viscosities of the components [18], which would explain our observations on the emulsions. We can explain the poor surfactant properties of crude whey this negative mix and also by the presence of substances antisurfactantes (lactose, salts, minerals, immunoglobulins .... etc..). The results of Wang et al., [54] show that the degree of damage to the serum protein molecules of BSA (bovine serum albumin) increases with increasing frequency and ultrasonic irradiation time, in fact, the regime cavitation is reached when the amplitude of the voltage applied to the triplet is such as to cause, within the medium to be studied, pressure changes having an amplitude greater than the pressure of liquid-vapor equilibrium, the temperature of the medium during the negative half-wave of pressure variation of the ultrasonic wave, there is the formation of small cavities filled with vapor, which cavities may explode during the positive half-cycle following the explosion of these small cavities is a violent phenomenon still poorly known: shock waves accompanied locally and instantaneously pressure surges and very high temperature, and the possibility of secondary chemical reactions, among these reactions, radical production free H + and OH- and the formation of H2O2 has been shown for a long time. Presence of hydrogen peroxide has been given as responsible for the destruction of many microorganisms. The study made by Rezek Jambrak et al., [49] on the functional properties of whey proteins (foaming properties) sonicated showed that these vary substantially according to the number of applied frequency and processing time fixed; best results are obtained at 40KHz after 15min but after 30min from the treatment the temperatures of the samples increases and decreases the electrical conductivity during processing. The best emulsions values that have marked the highest stability that result in the most inferior standard deviations are shown in the photos given by figure 2.

Fig. 2: Photos of emulsions of sweet almond oil (HA) in crude whey (Br) and sweet almond oil (HA) in crude whey sonicated for 5min (Br *), 10min (Br **) and 15min (Br ***) taken by a light microscope (Microscope photonics PHYWE, hund WETZLAR, GERMANY, GX10) at time t0 showing the influence of power’s ultrasound treatment of the dispersing phase on the average size and dispersion of droplets (spherical shapes) of sweet almond oil emulsion HA / crude whey (Br); case of HA / LSAas (7g / l of proteins: Φ = 0.8μm, SD = 16.26%), emulsion HA / crude whey treated (Br *); case of HA / LSDss (13g / l proteins: Φ = 1.1 μm, SD = 6.07%), emulsion HA / crude whey treated (Br **); case of HA / LSDss (13g / the proteins: Φ = 0.75μm, SD = 3.03%), emulsion HA / crude whey treated (Br ***) , case of HA / LSAas (7g / l of proteins: Φ = 1.4μm, SD = 9.38%).

Conclusion:

The present study used in applied research under optimal conditions (nature of whey, method of treatment, treatment time, type of protein ....) the stability of emulsions of crude wheys (acid and soft); this noble waste is discharged directly into the environment (case :Trefl, Sidi Saada industry of cheese, yellel, relizane, ALGERIA) causing severe water pollution of freshwater ecosystems. The results show that the physicochemical parameters of sweet almond oil are suitable for emulsification, the values of the physicochemical parameters of the crude soft whey are higher than those found in whey including crude acid whey: proteins, lactose, density and viscosity, but the ultrasonic treatment applied to the crudes wheys during 5,10 and 15min produced pH values stable against he changed the values of the temperature: 26-27 ° C after 5 min, 30-31 ° C after 10 minutes and 32-33 ° C after 15min. The statistical
study shows that the average size and dispersion of fat globules at time t0 depends on the protein composition of the dispersing phase (crudes wheys), the presence or absence of sodium caseinate as stabilizer, and the ratio of oil / whey namely emulsion HA / liquid whey (Br); case of HA / LSAas (7 g / 1 of proteins: Φ = 0.8μm, SD = 16.26%), in addition to the ultrasonic treatment of crudes wheys improved emulsifying behavior over time compared to untreated crudes wheys which emulsion stability depends on the nature of the crudes wheys (acid and sweet), the presence or absence of sodium caseinate as stabilizer, the oil / whey and whey exposure time of the power’s sonication, the best stabilities recorded for emulsions of crudes wheys treated and untreated by ultrasound are: emulsion HA / crude whey (Br); case of HA / LSAas (7g / l of proteins: Φ = 0.8μm, SD = 16.26%), emulsion HA / crude whey treated (Br *); case of HA / LSDss (13g / l of proteins: Φ = 1.1μm, SD = 6.07%), emulsion HA / crude whey treated (Br **); case of HA / LSDss (13g / l of proteins: Φ = 0.75μm, SD = 3.03%), emulsion HA / crude whey treated (Br ***); case of HA / LSAas (7g / l of proteins: Φ = 1.4μm, SD = 9.38%). The ability of emulsions studied does not necessarily translate into lowering the diameter of the fat globules at time t0, but indicates its stability over time. Looking ahead, we will work on the application of other treatment techniques to better understand the problem of valorization of whey in the emulsification.

List of abbreviations:

HA / LSAas: Emulsion type of sweet almond oil in acid whey without stabilizer
HA / LSAas: Emulsion type of sweet almond oil in acid whey with stabilizer
HA / LSDss: Emulsion of sweet almond oil in soft whey without stabilizer
HA / LSDas: Emulsion of sweet almond oil in soft whey with stabilizer
MS: Average stability
SD: Standard Deviation
Φ: Average diameter of the fat globules
G: Magnification
LSAB: Crude acid whey
LSDB: Soft crude whey

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