INCORPORATION OF SPIRULINA POWDER INTO PROCESSED MILK TO OBTAIN SEMIHARD CHEESE WITH THE AIM OF INCREASED NUTRITIONAL VALUE AND SENSORY CHARACTERISTICS

Roxana Ncoleta RAȚU[1], Ionuț Dumitru Veleșcu[1], Alina Narcisa POSTOLACHE[2], Sandu TALPĂ[1], Vlad Nicolae ARSENOAIA[1], Marius Giorgi USTUROI[1]

e-mail:roxana.ratu@uaiasi.ro

Abstract

Vital amino acids, essential fatty acids, carotenoids, and vitamins are all abundant in spirulina. The purpose of the study was to assess the effects of adding spirulina to processed cheese as a source of nutrients and color. To carry out this study, the milk was processed to obtain semi-paste cheese, making three batches as follows: the control batch (Lc) cheese without the addition of spirulina, the experimental batch 1 (Lexp-1) where the addition of 0.25% spirulina and experimental group 2 (Lexp-2) where 0.50% spirulina was added. For products enriched with Spirulina, we mention the fact that it was added to the milk after its pasteurization. Determinations were made on the finished product to establish the main physicochemical parameters after the cheese was kept for 30 days during maturation under specific conditions. The results obtained indicate increases in the protein level, therefore from 19.47% obtained in Lc to 19.87% in Lexp-1 and 20.27% in Lexp-2. Differences can also be noted in terms of total mineral content (ash), the value obtained for Lexp-2 being 3.32% higher than that obtained for Lc. Therefore, the results of this study highlight the fact that we can increase the nutritional value of a product, managing to come to consumers with a less conventional product.

Key words: cheese, quality, spirulina

Cheese is a product with very ancient origins, loved for its rich nutritional value, creaminess and specific aroma. It was discovered in ancient civilizations during the rise of agriculture and the domestication of sheep and goats for their milk. By chance, when the milk was left in the sun for a longer time, it "turned sour" and the protein components coagulated, becoming solid. When the liquid portion, or whey, was drained and removed, leaving only the solid curd, the farmers realized that this curd had a pleasant taste (Zheng X. *et al*, 2021).

Since then, cheese production has been experimented in countries all over the world, the differences being given by the types of milk from which the cheese was made, the period of aging of the cheese or the way of processing; all of which lead to products with unique textures and flavors. India is known for Paneer, a soft variety with a mild taste, Greece discovered Feta), a salty, crumbly cheese made from sheep or goat's milk, and Sardinia developed the hard, pungent cheese called Pecorino Romano (Thom C., Fisk W.W., 1918).

Cheese is a matured or unripened product obtained by coagulation of milk proteins, the action of rennet or another coagulant. Dehydration, lactic acid bacteria (LAB) fermentation and the addition of salt during cheese making increase its shelf life (Agarwal S. *et al,* 2006). The cheese contains the main milk protein casein, milk fat, mineral calcium phosphate, about 36-43% water, lactic acid and 1.5% salt for a hard cheese. Curd cut size, curd heating conditions and pressing influence moisture content and texture (Whetstine C. *et al*, 2007).

Cheese specialties are high-quality products that are consumed in limited quantities. These cheeses may have an exotic origin or a certain processing technique or design. Specialty cheeses are produced all over the world, with famous varieties coming from France, Italy, Mexico, the Middle East, Germany, Holland and Switzerland.

Cheese specialties also have different consistency and color, which can vary from soft to hard, from white to yellow/orange. Also, raw milk can vary - some are made from cow's milk, others from sheep's or goat's milk. There are even specialty cheeses made from milk combinations (mixed milks) (Whitehead W.E. *et al*, 1993).

Cheese can be classified into different groups depending on the type of milk, heat

[1] Iasi Uneversity of Life Science, Romania
[2] Research and Development Station for Cattle Breeding Dancu, Romania

treatment, type of coagulation, curd preparation method, water content in the finished product, fat content or method and degree of ripening (*table 1* and *figure 1*) all of which lead to obtaining countless varieties of cheese, types that consumers can enjoy.

Table 1

Major classes of cheeses

(Codex Alimentarius. General Standard for Cheese, 1999)

According to firmness		*According to milkfat content*	
MFFB (%)	**Designation**	**FDM (%)**	**Designation**
<51	Extra hard	≥ 60	Hight fat
49 - 56	Hard	45 - <60	Full fat
54 - 69	Firm/Semi-hard	25 - <45	Medium fat
>67	Soft	10 - <25	Partially skimmed
		<10	Schimmed

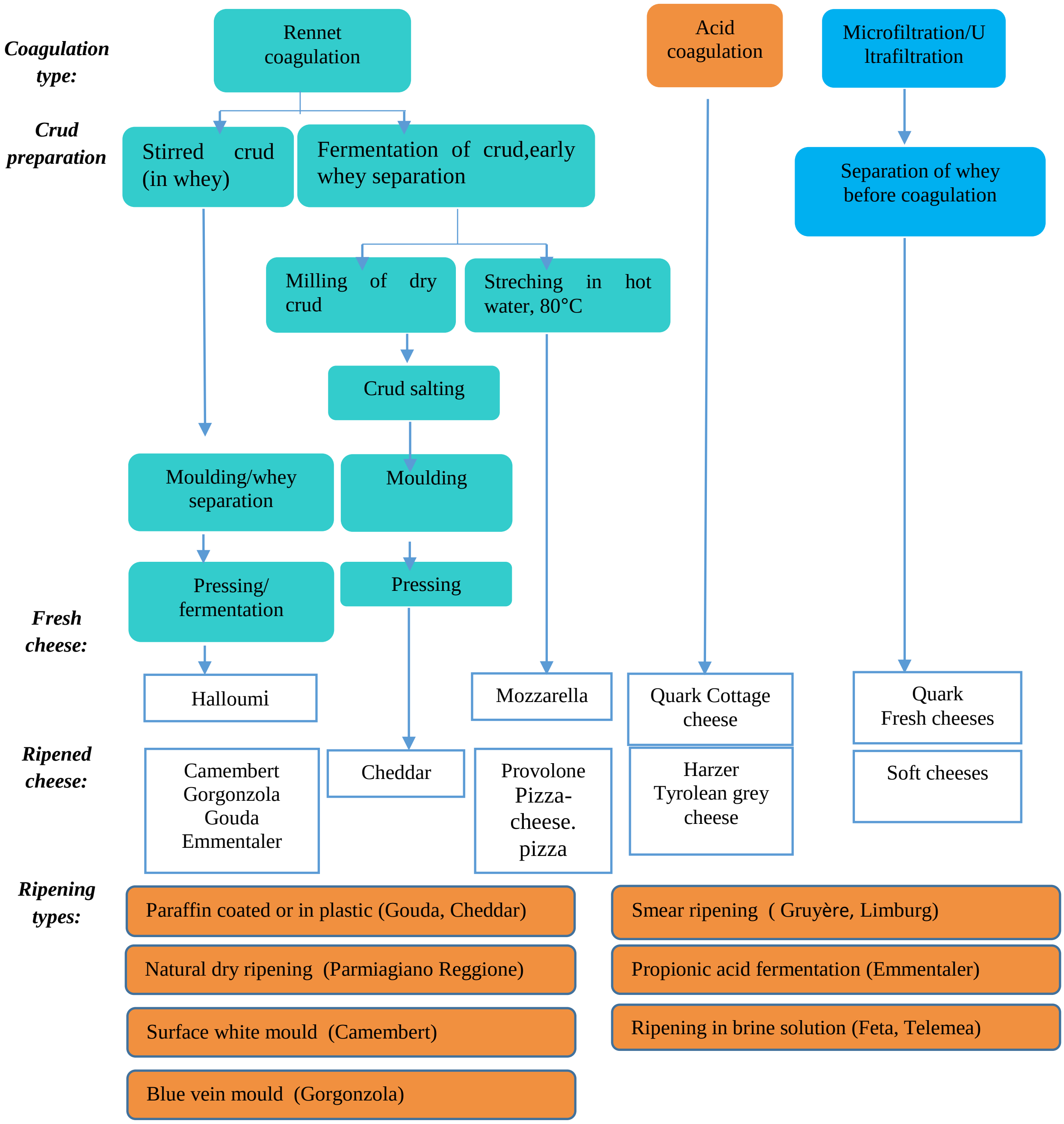

Figure 1 **Classification of cheese according to coagulation, crud preparation and ripening**

(after: Cheese-and-varieties-Part-2_-Cheese-styles-.pdf)

The group of dairy products in the semi-paste cheese category differs from the rest of the cheese assortments by the lower water content it has in its chemical composition. The low water content of the finished product is mainly due to the more intense processing of the coagulum and the application of the second heating stage, carried out at a moderate temperature range, followed by a pressing of the product.

The assortment of Holland-type semi-paste cheese is obtained from the processing of cow's milk, in spherical or parallelepiped form.

Because of its delicious flavor, palatable texture, and simplicity in preparation and consumption, processed cheese is very well-liked all over the world. Processed cheeses are made by chopping or shredding natural cheeses with varying levels of ripeness and combining them with emulsifying agents while heated, in a partial vacuum, or at room pressure, until a homogenous mass is created. Other components, such as powdered milk, stabilizers, preservatives, water, meat, fruit, and spices, among others, may be added in accordance with local law. Numerous research have looked at processed cheese that has been supplemented with Spirulina platensis as a nutrient source. There isn't much information available about using Spirulina maxima as an antioxidant and color source while making processed cheese. A multicellular, filamentous, photosynthetic cyanobacterium is spirulina (Arthrospira platensis). It is a good source of carbohydrates, pigments, iron, calcium, polyunsaturated fatty acids (PUFAs), essential amino acids, and vitamins E, C, and B12 (Markou G. *et al*, 2013; Golmakani M. *et al*, 2012). Spirulina possesses a variety of properties, including the ability to exert cytotoxic effects on human cancer cell lines (Flores Hernandez *et al*, 2017), hepatoprotective activity (Kepekçi R.A. *et al*, 2013), antioxidant activity (Alavi N. and Golmakani M., 2017), and anti-inflammatory effects (Kepekçi R.A. *et al*, 2013). (Pak W. *et al*, 2012) Additionally, Spirulina may play a part in the development of probiotic bacteria. In this study, S. maxima powder was added to processed cheese to boost its nutritional content and serve as a source of color and antioxidants. The goal was to ascertain how S. maxima powder affected processed cheese's quality traits after 30 days of storage at 12°C.

MATERIAL AND METHOD

Cheese making

To achieve the proposed goal, a quantity of 300 L of milk was processed as follows: 100 L of pasteurized milk to which 500 g of Spirulina in powder form was added, which represents a percentage of 0.5%, 100 L of milk with 250 g powder of Spirulina which represents a percentage of 0.25% and 100 L of which cheese was processed without addition (*figure 2*).

Figure 2. **Addition of spirulina in the volume of pasteurized and seeded milk**

The stages of the technological process were those specific to the assortment of semi-paste cheese, with the mention that for products with spirulina, the homogenization in milk was carried out after the pasteurization stage. Therefore, the main steps are: for each batch of milk that was processed, the filtering operation was carried out, after which the milk was pasteurized in the valve at a temperature of 60°C with maintenance for 30 minutes. To coagulate the milk, it was cooled to a temperature of 32°C after which the yeast of selected lactic bacteria (Mesophilic aromatic culture, type LD) was added. The ripening of milk lasts until the acidity of the milk increases by 0.8 - 1°T. In the milk, warmed to 32 - 35°C was added CHY-MAX M Liquid a standardized solution of pure chymosin, produced by the submerged fermentation of a vegetable substrate with the help of *Aspergillus niger* var. Awamori kept under controlled conditions (it is not found in the finished product), produced by CHR HANSEN.

Coagulation was carried out for 35–40 min. The curd was then cut longitudinally and transversally using knives to the size of a grain of corn, followed by heating under continued agitation up to a temperature of 42°C. Then followed the drying of the grain, which was done at the temperature of the second heating for 10-15 minutes. At the end of this process the curd was left for 10 min, then most of the whey was taken. The curd was then transferred to plastic rectangular moulds (figure 3) and pressed for 14 h using a pressure of 2–6 kg/ kg of cheese. During pressing the curd was turned over two or three times. The salting was done with a 12% brine by keeping the cheese for a period of 36-48 hours.

After removing from the brine pool, the pieces of cheese are left to swirl. During pressing the curd was turned over two or three times. Finally, the cheeses were ripened in rooms or cellars for 30 days at an average temperature of 12–14°C and at a relative humidity of 80–90%. The weight of the cheeses at the end of ripening varied between 1,5 and 1,8 kg, the yield being 9.8 L

Figure 3 **Filling the molds**

Cheese sampling

For each batch of cheese, samples were made up of milk and seven rectangular cheeses taken from the ripening places. The samples were taken to the laboratory under refrigeration at 4°C.

Chemical analysis

The chemical parameters of milkwere determined using a laboratory ultrasonic analyzer EKOMILK-Bond Total equipment that uses the principle of ultrasound transmittance through a resonance chamber in which the sample to be analyzed is located to measure the impedance changes of the sensor - detector produced by the oscillations of the wavelength of the ultrasound beam under the influence of the change in the proportions of the chemical components in the sample under study . the titratable acidity was determined by AOAC standard (Association of Official Analytical Chemists, 1990a) and the pH was measured with a WTW InoLab pH-meter.

The moisture (water) content of cheese is the loss in mass, expressed as a percentage by mass when the product is heated in an air oven at 102±2°C to constant mass (IS:2785:1979; Reaffirmed 1995). Dray matter content resulted from the difference, according to the relation: Day matter (%) = 100% – watter (%).

The fat content was determinated by Acid Digestion Method (AOAC, 2003) The fat content of the cheese in relation to the dry matter (FDM) was calculated according to the formula FDM (%) = (fat%/(100 - water))x100.

The Kjeldahl Method was used to determine the protein content in cheese. Because of its great precision and consistency, as well as its ease of use, Kjeldahl is presently the most widely used method for assessing nitrogen and protein levels in meals and feeds. The contemporary Kjeldahl process entails catalytically aided mineralization of organic matter in a boiling combination of sulfuric acid and sulfate salt at a temperature of 400°C in the digesting block. The biologically bound nitrogen is transformed to ammonium sulfate during the process. The ammonia is quantitatively steam distilled and measured by titration after alkalinizing the digested solution (IDF 20-1, ISO 8968-1 Second Edition 2014-02-01 Milk and milk products - Determination of nitrogen content; AOAC 991.20 Nitrogen (Total in Milk).

Ash was determined according to AOAC gravimetric method 935.42 (AOAC, 2000a). In all samples, pH was measured with an HI 99161 pH meter (Hanna Instruments, Weilheim, Germany) by direct insertion of a pH probe (FC2002; Hanna Instruments) into the cheese

The determination of sodium chloride was performed by the Mohr method, the principle of the method consisting of the precipitation of chlorides with silver nitrate solution in the presence of potassium chromate as an indicator (MANUAL OF METHODS OF ANALYSIS OF FOODS FOOD SAFETY –Milk and Mipk Products).

Sensorial evaluation

Five experts (aged 22 to 35) conducted a consumer acceptance test on the following dairy-related characteristics: sour taste, microalgae odor and taste, sweetness, bitterness, dairy flavor, crumbly texture, smoothness, color, and general acceptability. The use of dairy and fermented milk products was known to the assessors.

RESULTS AND DISCUSSIONS

The processed milk comes from the university's farm, a fact for which there were no differences in terms of the analysis part. Therefore, the milk was processed in three different steps to make the products that were the basis of the present study.

For these reasons, for the dry substance content, the average value was 12.66±0.04 for milk from Lc, 12.99±0.05 for that from Lexp-1 and 12.84±0.05% for that of Lexp-2. The fat content varied between 4.02±0.02% in the Lexp-2 group and 4.06±0.05%. The protein level data revealed mean values of 3.25±0.01% in the control group (Lc), 3.31±0.02% in the Lexp -1 group, and 3.31±0.01% to batch Lexp-2. Regarding the pH value, the average values were between 6.58 in the Lc group and 6.62 in the Lexp-1 group. Determinations were also made to determine the acidity of the milk, where the average values were 16.40±0.24°T in the milk distributed in the Lc group, 16.20±0.20°T in the Lexp-1 group, and 16 .00±0.32°T to that of the Lexp-2 batch (*table 2*).

Regarding the results obtained on the

finished products, we note that the addition of spirulina positively influenced the main chemical characteristics. Therefore, the dry matter content of group Lc was 56.67±0.20%, 56.70±0.20% in group Lexp-1, and 56.76±0.20% in group Lexp-2. Changes were also noted in terms of fat content, so in the Lc group the average value was 27.90±0.11%, 28.10±0.11% in the Lexp-1 group, and 28.35 ±0.11% at batch Lexp-2 (*table 3*).

Table 2

Chemical composition of milk

Specification	Milk use for normal cheese (Lc)	Milk use for cheese with SP 0.25% (Lexp-1)	Milk use for cheese with SP 0.50% (Lexp-2)
Moisture (%)	87.34±0.04	87.01±0.05	87.16±0.05
Dray matter (%)	12.66±0.04	12.99±0.05	12.84±0.05
Fat (%)	4.06±0.05	4.04±0.06	4.02±0.02
Protein (%)	3.25±0.01	3.31±0.02	3.31±0.01
pH	6.58±0.04	6.62±0.04	6.60±0.04
Acidity (°T)	16.40±0.24	16.20±0.20	16.00±0.32

Table 3

Chemical composition of cheese

Specification	Normal cheese (Lc)	Cheese with SP 0.25% (Lexp-1)	Cheese with SP 0.50% (Lexp-2)
Moisture	43.33±0.20	43.30±0.20	43.24±0.20
Dray matter (TS)(%)	56.67±0.20	56.70±0.20	56.76±0.20
Fat	27.90±0.11	28.10±0.11	28.35±0.11
FDM (%)	49.24±0.15	49.56±0.15	49.95±0.15
Protein (%TS)	19.47±0.14	19.87±0.14	20.27±0.14
Ash (%TS)	4.94±0.18	5.01±0.18	5.11±0.18
pH	5.19±0.02	5.17±0.01	5.13±0.01
Salt content (%)	1.90±0.03	1.93±0.03	1.97±0.03

Table 4

Sensorial evaluation

Specification	The weighting factor	Normal cheese (Lc)	Cheese with SP 0.25% (Lexp-1)	Cheese with SP 0.50% (Lexp-2)
External appearance	0.4	5 2	4 1.6	5 2
Color	0.2	4 0.8	4 0.8	5 1
Section apparance	0.8	5 4	5 4	5 4
Consistency	0.4	5 2	5 2	5 2
Smell	0.8	5 4	5 4	5 4
Taste	1.4	5 7	5 7	5 7
TOTAL		19.8	19.4	20

Regarding the protein level in the product that was the objective of this study, it can be seen that it increased simultaneously with the percentage of spirulina added. Therefore, in the product that does not contain spirulina, (Lc) the average value of the protein level was 19.47±0.14% in the cheese distributed in the Lexp-1 lot, where the addition of spirulina powder was 0.25 % the protein content was 19.87±0.14% and in the Lexp-2 group where the milk had an addition of 0.50% spirulina powder the protein level rose to a level of 20.27±0.14%. Differences were also noted regarding the ash content, therefore, for this qualitative parameter the values were 4.94±0.18% in the Lc batch, 5.01±0.18% in the Lexp-1 batch and 5.11±0.18% in the Lexp-2 group. Regarding the pH value after 30 days of ripening, values of 5.19±0.02 were recorded for the product distributed in the Lc batch, 5.17±0.01 for the Lexp-1 and 5.13±0.01 for the product from the Lexp-2 batch (*table 3*).

For the salt content, the average values obtained were 1.90±0.03% in the control group (Lc), 1.93±0.03% in the Lexp-1 group and 1.97±0.03 % to the Lexp-2 lot.

Regarding the sensory evaluation that was carried out by the point method, the total score for each variety analyzed was 19.8 in the control group (Lc), 19.4 for Lexp-1 and 20 points in the Lexp-2 group (*table 4*).

CONCLUSIONS

Proteins from microalgae biomass are thought to be a sustainable source that can supply the rising need for these biomolecules on a worldwide scale. One of the most nutrient-dense microalgae is spirulina, which can have up to 70% of its dry bulk made up of protein. As expected, the values of the quality chemical parameters were higher compared to those obtained in the control group (Lc). From a sensory point of view, the color in particular but also the taste can have a positive or negative impact on the consumers, although, as far as the taste is concerned, it is specific to matured cheese, the changes suffered by the addition of spirulina powder being slight.

REFERENCES

Agarwal S., Powers J.R., Swanson B. G., Chen S., and Clark S, 2006 - *Cheese pH, protein concentration, and formation of calcium lactate crystals*. J. Dairy Sci. 89:4144–4155.

Alavi N., Golmakani M-T., **2017** - *Antioxidant properties of whole-cell Spirulina (Arthrospira platensis) powder expressed in olive oil un- der accelerated storage conditions.* J Appl Phycol 29:2971–2978

AOAC (2003) AOAC Official Method 933.05, Fat in Cheese. Washington, DC: AOAC International.

AOAC (1980b). Hydrogen-Ion activity (pH). Horwitz, W. (Ed.), Official methods of analysis (p. 213). Washington: AOAC.

AOAC (2000a) 33.7.07 AOAC Official Method 935.42 Ash of Cheese. Gaithersburg, MD: AOAC International.

AOAC (1990a). Acidity in milk. Helrich, K. (Ed.), Official methods of analysis (vol. 2) (p. 805). Virginia: AOAC.

Carunchia Whetstine M. E., Luck P. J., Drake M. A., Foegeding E. A., Gerard P. D. and Barbano D. M., 2007 - *Characterization of flavor and texture development within large (291 kg) blocks of Cheddar cheese.* J. Dairy Sci. 90:3091–3109

Codex Alimentarius. *General Standard for Cheese. CXS 283-1978. Revised (1999). Amended (2006, 2008, 2010, 2013, 2018) (1973), Food and Agriculture Organization,* World Health Organization.

Dairy Federation, **2021** - *Cheese and Varieties Part II:Cheese Styles*, February.

Flores Hernandez FY, Khandual S, Ramírez López I.G., 2017 - *Cytotoxic effect of Spirulina platensis extracts on human acute leukemia Kasumi-1 and chronic myelogenous leukemia K-562 cell lines*. Asian Pac J Trop Biomed 7:14–19

Golmakani M-T., Rezaei K., Mazidi S., Razavi S.H., 2012 - *Effect of alter- native C_2 carbon sources on the growth, lipid, and γ linolenic acid production of Spirulina (Arthrospira platensis).* Food Sci Biotechnol 21:355–363

IDF Fractsheet 18/2021, *Cheese and Varieties Part II: Cheese Styles.*

ISO 8968-1:2014 [IDF 20-1:2014] Milk and milk products — Determination of nitrogen content — Part 1: Kjeldahl principle and crude protein calculation.

Kepekçi R.A., Polat S., Çelik A., Bayat N., Saygideger S.D., 2013 - *Protective effect of Spirulina platensis enriched in phenolic compounds against hepatotoxicity induced by CCl_4*. Food Chem 141:1972–1979.

Markou G., Angelidaki I., Nerantzis E., Georgakakis D., 2013 - *Bioethanol production by carbohydrate-enriched biomass of Arthrospira (Spirulina) platensis.* Energies 6:3937–3950

Pak W., Takayama F., Mine M., Nakamoto K., Kodo Y., Mankura M., Egashira T., Kawasaki H., Mori A., 2012 - *Anti-oxidative and anti- inflammatory effects of spirulina on rat model of non-alcoholic steatohepatitis*. J Clin Biochem Nutr 51:227–234

Thom C. and Fisk W.W., 1918 - *History and development of the cheese industry in America*. Pages 311–326 in The Book of Cheese. L. H. Bailey, ed. McMillan, New York, NY

Whitehead W.E., Ayres J.W., and Sandine W.E. **1993** - *A review of starter media for cheese making.* J. Dairy Sci. 76:2344–2353.

Xiaochun Zheng, Xuewei Shi, Bin Wang A., **2021** - *Review on the General Cheese Processing Technology, Flavor Biochemical Pathways and the Influence of Yeasts in Cheese*. *Frontiers in Microbiology*.