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OPTIMIZING THE LIPID EXTRACTION PROCESS FROM *ARTEMIA* CYSTS. A COMPARATIVE ANALYSIS

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ОПТИМИЗАЦИЯ ПРОЦЕССА ЭКСТРАКЦИИ ЛИПИДОВ ИЗ ЦИСТ ARTEMIA: СРАВНИТЕЛЬНЫЙ АНАЛИЗ

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Tayanch so‘zlar: artemia, sista, lipid ekstraksiyasi, Folch usuli, Bligh & Dyer, Soxhlet, akvakultura, optimallashtirish

Rezyume. Artemia (sho‘r suv qisqichbaqasi) sistalari akvakultura sohasida baliq va qisqichbaqasimonlar lichinkalari uchun asosiy jonli oziqa manbai bo‘lib, ularning oziqaviy qiymati asosan lipid miqdori va yog‘ kislotalari tarkibi bilan belgilanadi. Lipidni to‘g‘ri va to‘liq ajratib olish usuli ilmiy natijalarning aniqligiga bevosita ta'sir qiladi, biroq adabiyotda turli mualliflar turli ekstraksiya usullaridan (Folch, Bligh & Dyer, Soxhlet, sulfofosfovanillin) foydalanadi, bu esa natijalarni bir-biriga solishtirishni qiyinlashtiradi. Ushbu maqolada 1985–2019 yillar oralig‘ida chop etilgan 11 ta ilmiy manbadan (turli geografik hudud, shtamm va rivojlanish bosqichlari bo‘yicha jami 47 ta ma'lumot qatori) olingan umumiy lipid miqdori va ekstraksiya usullari qiyosiy tahlil qilindi. Natijalar shuni ko‘rsatdiki, Folch usuli eng ko‘p qo‘llanilgan va eng barqaror natija beruvchi oltin standart hisoblanadi, biroq quritilgan namunalar uchun Bligh & Dyer usuli lipidni to‘liq ajratib ololmasligi (Soxhlet bilan solishtirilganda 30-45% past natija) aniqlandi. Shu asosda Artemia sistalaridan lipidlarni ajratib olishni optimallashtirish bo‘yicha bosqichma-bosqich protokol taklif etildi.

Ключевые слова: артемия, циста, экстракция липидов, метод Фольча, Блай и Дайер, Сокслета, аквакультура, оптимизация

Резюме. Цисты артемии (артемии) являются основным источником живого корма для личинок рыб и ракообразных в аквакультуре, а их питательная ценность в основном определяется содержанием липидов и составом жирных кислот. Правильный и полный метод экстракции липидов напрямую влияет на точность научных результатов, но в литературе разные авторы используют разные методы экстракции (Фольча, Блай и Дайера, Сокслета, сульфофосфованиллина), что затрудняет сравнение результатов между собой. В данной статье представлен сравнительный анализ общего содержания липидов и методов экстракции, полученных из 11 научных источников (всего 47 строк данных по различным географическим регионам, штаммам и стадиям развития), опубликованных в период с 1985 по 2019 год. Результаты показали, что метод Фольча является наиболее широко используемым и наиболее надежным «золотым стандартом», но для высушенных образцов метод Блай и Дайера оказался неполным в экстракции липидов (на 30–45% ниже, чем метод Сокслета). На этой основе был предложен пошаговый протокол оптимизации экстракции липидов из цист артемии.

Key words: artemia, cyst, lipid extraction, Folch method, Bligh & Dyer, Soxhlet, aquaculture, optimization.

Summary. Artemia (brine shrimp) cysts are the primary live-feed source in aquaculture for fish and crustacean larvae, and their nutritional value is largely determined by their lipid content and fatty-acid composition. The method used to extract lipids has a direct impact on the accuracy of scientific findings, yet different authors in the literature employ different extraction techniques (Folch, Bligh & Dyer, Soxhlet, sulfophosphovanillin), which complicates cross-study comparison. In this paper, the total lipid content and extraction methods reported in 11 scientific sources published between 1985 and 2019 (covering various geographic regions, strains, and developmental stages, totaling 47 data rows) were comparatively analyzed. The results showed that the Folch method is the most widely used and the most consistently reliable, functioning as a "gold standard," whereas the Bligh & Dyer method was found to fail to fully extract lipid from dried samples (yielding 30–45% lower results compared with Soxhlet cross-checks). On this basis, a step-by-step protocol is proposed for optimizing lipid extraction from Artemia cysts.

Introduction. Members of the genus Artemia (brine shrimp) are distributed across numerous saline water bodies worldwide, and their dried cysts serve as the primary means of feeding fish and crustacean larvae in the aquaculture industry. The nutritional value of cysts and the nauplii hatched from them depends first and foremost on their lipid content and, within it, the amount of highly unsaturated fatty acids (EPA, DHA). Accurate and complete extraction of Artemia lipids is therefore one of the key factors determining the quality of scientific research in this field. A review of the literature shows that different researchers use different methods to extract lipids - ranging from the classic Folch [12] chloroform:methanol extraction to Bligh & Dyer [13], Soxhlet extraction, and the sulfophosphovanillin colorimetric method. Each of these methods differs in operating principle, sensitivity, and efficiency depending on sample condition (fresh, frozen, or dried), meaning that sharply divergent lipid values can be obtained even for the same geographic region or strain. The aim of this paper is to combine the total lipid content and extraction methods reported for Artemia cyst samples (as well as nauplius, metanauplius, and decapsulated cyst samples) across 11 scientific sources published by various authors between 1985 and 2019 into a single unified table for comparative analysis, to identify the strengths and weaknesses of each method, and, on this basis, to develop scientifically grounded recommendations for optimizing lipid extraction from Artemia cysts.

Materials and methods. Source selection criteria. For the analysis, 11 peer-reviewed scientific papers were selected that quantitatively report the total lipid content of Artemia sp. cysts (as well as nauplius/metanauplius stages) and clearly describe the extraction method used. The sources covered geographically diverse regions (Spain, Algeria, the Aral Sea, Iran, Tunisia, the USA), which allowed for broader generalization of the findings.

Comparative analysis methodology. The following parameters were extracted from each source: author(s), year, journal, sample type/stage, total lipid content (mean ± standard deviation), and the extraction/determination method used. All data were entered into a single unified table (11 sources, 47 rows) (Section 3, Table 1). Extraction methods were grouped into four main categories: (1) Folch [12] chloroform: methanol; (2) Bligh & Dyer [13]; (3) Soxhlet extraction; (4) sulfophosphovanillin colorimetric method. For each method, the sample condition (moist/frozen/dried), frequency of use (number of papers employing it), and relative efficiency (where available – based on studies that directly compared several methods on the same sample, notably Liou & Simpson, [7] were compared. No statistical processing was applied – this is a primary (descriptive) meta-analysis, the purpose of which is to qualitatively compare methods and identify directions for optimization.

Results. Summary table. Table 1 Presents all 47 data rows compiled from the 11 sources - total lipid content together with the method used to determine it.

Table 1. **All information on total lipid levels and their determination from 11 sources.**

Source (paper)	Sample / stage / strain	Total lipid content	Extraction / determination method	Year
Navarro, Amat, Sargent [1]	GSL – freshwater type (Great Salt Lake)	9.2 ± 0.2 % (dry weight)	Folch (1957) extraction + gravimetric	1993
Navarro, Amat, Sargent [1]	LMT – marine type (La Mata, Spain)	17.9 ± 0.4 % (dry weight)	Folch (1957) extraction + gravimetric	1993
Landau & Riehm [2]	San Francisco strain	2.05 ± 1.21 % (of body weight)	Sulfophosp-hovanillin method	1985
Landau & Riehm [2]	Colombia strain	1.04 ± 0.55 % (of body weight)	Sulfophosp-hovanillin method	1985
Landau & Riehm [2]	Australia strain	2.71 ± 0.96 % (of body weight)	Sulfophosp-hovanillin method	1985
Landau & Riehm [2]	Utah strain	7.03 ± 1.10 % (of body weight)	Sulfophosp-hovanillin method	1985
Navarro, Amat, Sargent [3]	Cyst	16.7 ± 0.3 %	Folch (1957), gravimetric + GC	1991
Navarro, Amat, Sargent [3]	Nauplius	22.1 ± 0.3 %	Folch (1957), gravimetric + GC	1991
Navarro, Amat, Sargent [3]	Metanau-plius	20.3 ± 1.1 %	Folch (1957), gravimetric + GC	1991
Navarro, Amat, Sargent [3]	Decapsu-lated cyst	17.7 ± 0.1 %	Folch (1957), gravimetric + GC	1991
Navarro, Amat, Sargent [4]	12 Spanish populations (cysts)	9.5–17.85 % range	Presented graphically; exact method not specified	1992

Ama-rouayache et al. [5]	Chott Marouane	13.79 %	Folch (1957)	2017
Ama-rouayache et al. [5]	Sebkha Ez-Zemoul	18.23 %	Folch (1957)	2017
Ama-rouayache et al. [5]	El-Bahira	16.48 %	Folch (1957)	2017
Ama-rouayache et al. [5]	Sidi Bouziane (highest)	24.55 %	Folch (1957)	2017
Ama-rouayache et al. [5]	Bethioua (lowest)	7.78 %	Folch (1957)	2017
Navarro, Amat, Sargent [6]	Bonmati (coastal)	19.42 ± 0.14 %	Folch (1957), gravimetric	1992
Navarro, Amat, Sargent [6]	La Mata (coastal)	17.80 ± 0.52 %	Folch (1957), gravimetric	1992
Navarro, Amat, Sargent [6]	La Trinidad (coastal, lowest)	11.48 ± 0.68 %	Folch (1957), gravimetric	1992
Navarro, Amat, Sargent [6]	Los Hermanos (coastal)	15.57 ± 0.82 %	Folch (1957), gravimetric	1992
Navarro, Amat, Sargent [6]	Olmeda. Gerri (7 inland populat.)	9.52–16.70 % range	Folch (1957), gravimetric	1992
Liou & Simpson [7]	Freshly hatched nauplii (control)	179 ± 9 mg/g	Bligh & Dyer (1959)	1989
Liou & Simpson [7]	Freeze-dried	176 ± 8 mg/g	Bligh & Dyer (1959)	1989
Liou & Simpson [7]	Vacuum-dried	163 ± 5 mg/g	Bligh & Dyer (1959)	1989
Liou & Simpson [7]	Hot-air dried	134 ± 6 mg/g	Bligh & Dyer (1959)	1989
Liou & Simpson [7]	Freeze-hot-air dried (cross-check)	257–258 mg/g	Soxhlet extraction (supplementary method)	1989
Berdim-betova et al. [8]	Cyst – free lipid (FL)	11.30 % (moist) / 12.13 % 12.14 (dry)	Petroleum-ether Soxhlet extraction (24 h)	2019
Berdim-betova et al. [8]	Cyst - bound lipid (BL)	2.23 % (NL 0.65 + GL 0.34 + PL 1.24)	$CHCl_3$–MeOH extraction + silica-gel CC	2019
Berdim-betova et al. [8]	Total lipid (FL + BL)	14.36 %	Sum of both methods	2019
Peykaran Mana et al. [9]	Cyst - Meyghan Lake	7.67 ± 0.7 %	Soxhlet method	2014
Peykaran Mana et al. [9]	Cyst - Maharlu Lake	6.62 ± 0.6 %	Soxhlet method	2014
Peykaran Mana et al. [9]	Cyst - Urmia Lake	7.15 ± 0.6 %	Soxhlet method	2014
Peykaran Mana et al. [9]	Instar I nauplius - Meyghan	12.42 ± 3.1 %	Soxhlet method	2014
Peykaran Mana et al. [9].	Instar I nauplius - Maharlu (highest)	18.60 ± 1.1 %	Soxhlet method	2014
Peykaran Mana et al. [9]	Instar I nauplius - Urmia	12.38 ± 3.5 %	Soxhlet method	2014
Peykaran Mana et al. [9]	Decapsulated cyst - Urmia (highest)	11.87 ± 2.1 %	Soxhlet method	2014
Peykaran Mana et al. [9]	Nauplius from decap. cyst - Maharlu	16.62 ± 2.1 %	Soxhlet method	2014
Ben Naceur et al. [10]	ZAR (lowest)	12.1 ± 0.4 %	Folch (1957)	2012
Ben Naceur et al. [10]	BK (highest)	20.2 ± 0.9 %	Folch (1957)	2012
Ben Naceur et al. [10]	11 populations	16.8–19.1 %	Folch (1957)	2012
Ben Naceur et al. [10]	(GSL, reference)	18.0 ± 0.9 %	Folch (1957)	2012
Ben Naceur et al. [11]	ADH 02 (2002, lowest)	16.2 ± 0.8 %	Folch (1957)	2010
Ben Naceur et al. [11]	ADH 03 (2003, highest)	18.3 ± 0.5 %	Folch (1957)	2010
Ben Naceur et al. [11]	ADH 04 (2004)	17.9 ± 1.2 %	Folch (1957)	2010
Ben Naceur et al. [11]	ADH 05 (2005)	17.7 ± 1.1 %	Folch (1957)	2010
Ben Naceur et al. [11]	ADH 06 (2006)	16.9 ± 0.5 %	Folch (1957)	2010
Ben Naceur et al. [11]	ADH 07 (2007)	17.4 ± 0.6 %	Folch (1957)	2010

Comparative efficiency of extraction methods. Table 2 summarizes the four main extraction methods – their frequency of use, suitable sample condition, relative efficiency, and main limitations.

Table 2. **Comparative efficiency of extraction methods**

Extraction method	No. of papers	Sample condition	Relative efficiency	Main limitation
Folch et al. chloroform: methanol (2:1)	8/11	Fresh/ frozen, decapsulated cyst	High, reproducible (gold standard)	Requires large volumes of toxic solvent, time-consuming
Bligh & Dyer	1/11	Dried biomass (various methods)	High in moist/fresh samples, underestimates in dried samples	Solvent penetration is hindered by tissue "shrinkage" during drying-30-45% lower yield compared with Soxhlet
Soxhlet extraction	3/11	Dried /powdered cyst, nauplius	Highest and Most complete (due to heat + prolonged contact)	High temperature may increase the risk of PUFA oxidation; time-consuming (hours)
Sulfophosphovanillin (colorimetric)	1/11	Dried/ freeze-dried cyst	Fast, requires little sample	Not directly gravimetric - depends on a cholesterol standard, sensitive to lipid-class composition, prone to error

Key findings. Total lipid content across all sources ranged from 1.0% (Colombia strain, Landau & Riehm, [2] to 24.6% (Sidi Bouziane, Amarouayache et al., [13]) – a difference attributable to geographic location, strain type, and, importantly, the extraction method used. In 8 of the 11 sources (73%), the Folch [12] method was used as the primary or supplementary method – confirming its status as the "gold standard. The direct comparison conducted by Liou & Simpson [5] yielded the most important methodological finding: in dried samples, the Bligh & Dyer [13] method gave 30-45% less lipid than Soxhlet extraction (134-176 mg/g versus 257-258 mg/g), which was attributed to the drying-induced "shrinkage" of cell tissue that prevents the solvent from fully penetrating. The sulfophosphovanillin colorimetric method (Landau & Riehm, [2]), while fast and requiring little sample, produced markedly lower absolute values (1.0-7.0%) than the other gravimetric/GC-based methods – likely related to the method's dependence on a cholesterol standard and its sensitivity to lipid-class composition. The two-stage approach (free + bound lipid) used by Berdimbetova et al. [8] yielded the most complete lipid profile, but this method is considerably longer and more complex, making it unsuitable for rapid screening.

Discussion. The findings once again confirm the absence of a single standardized protocol for extracting lipids from Artemia cysts. This situation makes direct comparison of results across studies difficult and introduces uncertainty into quality control in aquaculture practice. Based on the analysis, the following directions for optimization are proposed:

Choosing a method according to sample condition. For fresh or freeze-dried cyst/nauplius samples, the Folch [12] or Bligh & Dyer [13] methods provide sufficiently complete extraction, since the cellular structure remains intact and the solvent can penetrate the tissue freely. Conversely, for hot-air-dried or long-stored dry samples, Soxhlet extraction (or an extended-contact-time Folch protocol) is recommended, since in such samples the cell membrane has "shrunk" and simple cold-extraction methods cannot fully release the lipid.

Proposed optimized step-by-step protocol.

Step 1 (sample preparation): hydrate cysts in seawater → decapsulate following the method of Bruggeman et al. (removal of the chorion layer) → rinse in distilled water and dry gently (avoid hot-air drying beyond 48 hours, since this can cause up to 25% lipid loss, Liou& Simpson, [7].

Step 2 (extraction): extract using the Folch et al. [12] method in chloroform:methanol (2:1, v/v) with 0.01% BHT antioxidant added, under a nitrogen atmosphere (to prevent oxidation); if the sample has already been thoroughly dried, supplementing with a Soxhlet stage (or extending contact time to at least 6–8 hours) is recommended.

Step 3 (quantification): gravimetric weighing (on an analytical balance, ±0.1 mg precision) should be used as the primary method, while colorimetric methods such as sulfophosphovanillin should be used only for rapid screening, with subsequent gravimetric confirmation.

Step 4 (quality control): confirmation using at least two independent methods (e.g., Folch + Soxhlet) is recommended for each batch, especially when analyzing dried samples – as shown by Liou & Simpson [7], this allows methodological errors (of up to 30-45%) to be detected.

Step 5 (storage): store the extracted lipid at −20°C under a nitrogen atmosphere with BHT antioxidant in chloroform:methanol (2:1) solution – this prevents oxidation and quality degradation [1].

Limitations. This analysis relies on secondary (literature-based) data, and therefore the exact experimental conditions (temperature, time, sample mass) used in the original studies were not fully controlled for. Future studies that directly compare all four methods on the same Artemia batch (with statistical validation) would further refine the optimal protocol.

Conclusion. The comparative analysis conducted on the basis of 11 scientific sources shows that the Folch method remains the most reliable and widely used technique for extracting lipids from Artemia cysts, although it must be adapted to sample condition (moist versus dried). It was found that the Bligh & Dyer method yields significantly lower results for dried samples (30–45% lower compared with Soxhlet), demonstrating the critical effect of the drying process on extraction efficiency. The proposed five-step optimized protocol (preparation → adapted extraction → gravimetric confirmation → dual-method quality control → antioxidant-protected storage) can help improve the accuracy and reproducibility of results in Artemia lipid research, as well as improve the comparability of findings across different studies.

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