## International Platform of Registered Systematic Review and Meta-analysis Protocols



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# Cocoa Fermentation Systems and Their Influence on Process Efficiency and the Quality of Its Derivatives: A Systematic Review

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#### ADMINISTRATIVE INFORMATION

Support - CAPES and Fapesp.

Review Stage at time of this submission - The review has not yet started.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 14 July 2023 and was last updated on 14 July 2023.

#### **INTRODUCTION**

**eview question / Objective** P - Cocoa fruits; I - Fermentation systems: Spontaneous; Induced (starters, enzymes, incubation); Equipment; C - Unfermented cocoa fruits; O - Better quality of the products obtained; Reduction of process cost (including labor); Increased reproducibility. Research question: What is the influence of fermentation systems on process efficiency and quality of derivatives?

**Rationale** Although fermentation is notoriously one of the fundamental post-harvest stages of cocoa, the literature still has gaps with regard to the effects of different fermentation systems on the quality of the final product and process efficiency in various production contexts. In addition, the quality of fermented cocoa almond is a relevant factor for value addition and economic competitiveness of producers, as it is a raw material for the chocolate market that requires criteria related to sustainability and sensoriality. In this way, a systematic review on the subject can contribute to the identification of these gaps and to the advancement of technical-scientific knowledge about cocoa fermentation, providing significant information to producers, researchers, and decisionmakers.

**Condition being studied** The condition of interest for this systematic review is the cocoa fermentation process, which is a key step in the production of high-quality almonds. The review will address the different cocoa fermentation systems already studied and used in cocoa-producing regions, including spontaneous fermentation, induced fermentation and fermentation carried out in equipment built for this purpose. Thus, the systematic review will focus on comparing the effects of fermentation systems on the quality of the products obtained, reproducibility and production costs.

### **METHODS**

Search strategy The sources that will be researched to carry out this systematic review include: PubMed/Medline

Scopus

Web Of Science

Food Science and Technology Abstracts (FSTA) CABI

Agricola

SciELO

Brazilian Digital Library of Theses and Dissertations (BDTD)

Google Scholar

The research will be restricted to articles published in English, Portuguese, French, Spanish, and German, without restriction of publication period. The searches will be remade before the final analysis to ensure that all relevant evidence is included.

Example of search strategy for PubMed/MEDLINE: (cocoa OR cacao OR "Theobroma cacao"[Mesh]) A N D (ferment\*[Title/Abstract] OR fermentation\*[Title/Abstract] OR "microbial fermentation"[Mesh] OR "fermentation process"[Mesh]) AND ("product quality"[Mesh] OR "process efficiency"[Mesh] OR "productivity"[Mesh] OR "yield"[Mesh] OR "product yield"[Title/Abstract] OR "quality control"[Mesh] OR "quality assessment"[Title/ Abstract]).

Participant or population The "population" of this systematic review will be cocoa fruits, with an emphasis on seeds, regardless of the place or producing region, age of the cocoa plants, or soil type. The review will consider studies investigating different fermentation systems, including spontaneous, induced methods or mechanical systems in relation to traditional and consolidated processes, or even the absence of fermentation. Studies that have evaluated the contents of phenolic compounds, flavor precursors, volatile compounds, color of fermented cocoa beans, microbial growth dynamics, and enzymes will be included. Studies published in books, scientific journals, theses and dissertations, patents generated on the subject, as long as they meet the inclusion criteria, will be considered. The review will not include studies that focus solely on investigating other stages of cocoa processing, such as drying, roasting, grinding and conching. Studies dealing with the fermentation of foods or plants other than cocoa trees will be excluded.

**Intervention** The "population" of this systematic review will be cocoa fruits, with an emphasis on seeds, regardless of the place or producing region, age of the cocoa plants, or soil type. The review will consider studies investigating different fermentation systems, including spontaneous, induced methods or mechanical systems in relation to traditional and consolidated processes, or even the absence of fermentation. Studies that have evaluated the contents of phenolic compounds, flavor precursors, volatile compounds, color of fermented cocoa beans, microbial growth dynamics, and enzymes will be included. Studies published in books, scientific journals, theses and dissertations, patents generated on the subject, as long as they meet the inclusion criteria, will be considered. The review will not include studies that focus solely on investigating other stages of cocoa processing, such as drying, roasting, grinding and conching. Studies dealing with the fermentation of foods or plants other than cocoa trees will be excluded.

**Comparator** For this review on cocoa fermentation systems, the comparison alternatives include different methods of cocoa fermentation, different varieties of cocoa, different environmental and storage conditions, or even absence of fermentation. Some possible comparisons: Different cocoa fermentation systems: comparing the effects of fermentation in boxes, baskets, bags or other containers on product quality and process efficiency. Different periods of fermentation. Spontaneous versus starter culture-induced fermentation. Spontaneous fermentation versus the use of enzymes. Influence of cocoa pulp contents on fermentation. Influence of postharvest fruit storage. Controlled fermentation equipment/systems. Fermentation versus no fermentation.

**Study designs to be included** Primary studies, including experimental and observational studies, assessing the effectiveness of cocoa fermentation systems in terms of product quality and process efficiency, as well as patents filed on the proposed topic.

Eligibility criteria Based on the PICO elements, the eligibility criteria for this systematic review are: Population/Problem: Studies that have investigated cocoa fruits in order to ensure that the results are applicable specifically to this product. Intervention: Fermentation systems: Spontaneous, Induced (starters, enzymes, incubation), Equipment. Comparator: unfermented cocoa seeds. Outcome/Results: Quality of fermented cocoa beans, as this is the main result of interest of this review because it is an important indicator of the effectiveness of the different fermentation systems. Another result of interest is process efficiency, as this can affect production on a commercial scale. In addition to these, the additional eligibility criteria included are: Studies published in Portuguese, English, Spanish, French, and German. Studies available entirely in databases to which the State University of Campinas has access, or those with free access. Only studies that pass the risk of bias analysis and/or the quality assessment will be included.

**Information sources** Databases to which the State University of Campinas has access, or those with free access, including grey literature, as: PubMed/Medline, Scopus, Web Of Science, Food Science and Technology Abstracts (FSTA), CABI, Agricola, SciELO, Brazilian Digital Library of Theses and Dissertations (BDTD), Google Scholar. In case of insufficient or unclear information, all attempts to contact the authors will be made.

**Main outcome(s)** The pre-specified primary results for the systematic review on cocoa fermentation systems are:

1. Quality of the final product, measured through sensory and sensomics evaluation, presence of specific volatile compounds and quality methods of cocoa and chocolate derivatives (according to specific legislation of the countries).

2. Efficiency of the fermentation process, measured by the presence of specific volatile compounds, cutting test and other analytical methods used for cocoa classification (humidity, pH and acidity, among others).

3. Impact of fermentation systems on the chemical composition of the products obtained, including polyphenols and aromatic compounds.

Additional outcome(s) Pre-specified secondary outcomes of the systematic review:

1. Variations in post-harvest methods and their effects on the efficiency of the fermentation process and quality of the products obtained.

2. Impact of climatic and environmental conditions on the efficiency of the fermentation process and quality of the products obtained.

3. Evaluation of the effects of the induction of the fermentation process (addition of ingredients, starter cultures, enzymes) on the efficiency of the fermentation process and quality of the products obtained.

4. Identification of knowledge gaps in the literature and recommendations for future research on cocoa fermentation.

**Data management** The data will be recorded in an Excel spreadsheet or Google Sheets, or in a free systematic review software. All steps of the systematic review process will be recorded in a protocol that will be made publicly available. A detailed record of all eligibility criteria applied, the source of included studies and data extraction will be maintained and made publicly available.

Quality assessment / Risk of bias analysis The bias risk assessment method for this systematic review will follow the guidelines of tools such as the Quality Assessment Tool for Quantitative Studies (QATS), or others that better fit the types of studies found. The assessment will be done at the study level.

The reviewers involved in the quality assessment will be two, who will evaluate the studies independently. Disagreements between judgments will be resolved through discussion, consensus among reviewers and/or consultation with a third reviewer if necessary.

The results of the risk of bias assessment will be used as eligibility criteria, to avoid potential limitations of the studies included in the systematic review. A sensitivity analysis will be made based on, among other factors, the risk of bias assessment to assess the robustness of the results of the systematic review.

**Strategy of data synthesis** Only studies that are considered of high quality and low risk of bias will be included in the data synthesis. Specific criteria will be used for the selection of studies, such as the presence of complete information on the fermentation systems used, the methodology used, and the measurement of results. The data that will be synthesized include results of product quality and process efficiency, such as content of compounds of interest, flavor quality, fermentation time, process yield, among others. Measures of summary effect will be presented as weighted averages or odds ratios, as appropriate.

Meta-analysis methods will be used if there are sufficient studies with homogeneous data (n=3), to generate a general estimate of the fermentation system's effect on the product's quality and on the efficiency of the process. The formal method of combining data from individual studies will be the random effects meta-analysis. This method allows the weights of the individual studies to vary and, at the same time, incorporates the variability between studies into the overall estimate of the effect. Appropriate statistical models will be used to estimate heterogeneity between the studies included in the meta-analysis.

A narrative synthesis of the findings from the studies included in this review will be conducted to summarize and explain the findings with a qualitative approach. Given the complexity of the forms of evaluation about cocoa fermentation systems and their effectiveness in terms of product quality and process efficiency, a quantitative approach may not be appropriate. In order to standardize and systematize the approach, the guidelines of Synthesis Without Meta-analysis (SWiM) for systematic reviews (CAMPBELL et al., 2020) will be followed, addressing the proposed items whenever applicable. Studies with several components will be categorized according to the main component, that is, the component that the authors primarily emphasized.

**Subgroup analysis** Subgroup investigation will be performed to assess whether the efficacy and quality of the cocoa fermentation product are affected by specific factors such as geographic region, cocoa variety, fermentation system, drying method, fermentation process duration and batch size. These factors have been identified as potential effect modifiers, which can affect the relationship between cocoa fermentation systems and their results.

Definitions of subgroups:

1.Geographic region: will include studies that evaluated cocoa fermentation systems in different geographic regions such as South America, Central America, Africa, and Asia.

2.Type of cocoa: will include studies that evaluated different groups of cocoa, such as Forastero, Criollo and Trinitário and varieties.

3.Type of fermentation: This will include studies that have evaluated different fermentation systems, such as box fermentation, heaped-in pile fermentation jute sacks fermentation or equipment designed for fermentation.

4.Drying method: will include studies that evaluated different drying methods such as sun drying and artificial drying.

5.Fermentation Process Duration: This will include studies that evaluated different fermentation process durations, such as 3, 5 or 7 days.

6.Batch size: will include studies that evaluated different batch sizes, such as small (up to 100 kg of cocoa) and large (more than 100 kg of cocoa).

Planned analytical approach: stratified analysis of the studies. A descriptive analysis of each subgroup to assess the heterogeneity in each subgroup and a comparison between the subgroups using heterogeneity tests and metaregression to assess the association between the subgroups and cocoa fermentation results. A pvalue <0.05 will be considered statistically significant.

**Sensitivity analysis** A sensitivity analysis will be performed to assess the strength of the metaanalysis results. Subgroup analyses will be performed to explore possible sources of heterogeneity between studies, such as differences in the type of cocoa, fermentation time, type of fermentation system used, among others. Sensitivity analysis will be performed to ensure the robustness and reliability of the systematic review results. 1. Exclusion of studies with high risk of bias or low methodological quality;

2. Exclusion of studies that do not meet predefined inclusion/exclusion criteria;

3. Analysis of specific subgroups of studies or participants, such as studies with different types of intervention or participants with specific characteristics;

4. Use of different statistical methods to synthesize the data, such as fixed and random effect models;

5. Analysis of different thresholds of methodological quality, such as low quality or risk of moderate bias.

The results of each sensitivity analysis will be reported and compared with the results of the main analysis.

**Language restriction** Portuguese, English, Spanish, French, and German.

Country(ies) involved Collaborations from Brazil.

**Other relevant information** CAMPBELL, M. et al. Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline BMJ 2020;368:I6890. DOI: https://doi.org/10.1136/ bmj.I6890.

CAPES and Fapesp (Process 2022/10179-7).

**Keywords** Cacao; Theobroma cacao; Fermentation; Fermentation systems; Processing; Product quality; Process Efficiency; Production methods; Improvement interventions; Sensory analysis; Flavor Profile.

**Dissemination plans** The results of this systematic review will be made publicly available for free or through purchase or subscription.

#### **Contributions of each author**

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